

BOOKS RECEIVED

THE BLIND: THEIR CONDITION AND THE WORK BEING DONE FOR THEM IN THE UNITED STATES, by Harry Best, Ph. D., 764 pages, The Macmillan Company, New York, 1919.

Foreword. In the present study the field of inquiry in respect to the blind has been limited to the United States, except in so far as an account is necessary of the operations in foreign countries in the way of affording instruction to blind children and of devising a system of raised print, as an introduction to the work in this country. References are accordingly only to American sources, save as to a restricted number of publications in England dealing with the two subjects mentioned, with popular conceptions regarding the blind, and occasionally with other matters.

EMBRYOLOGY OF THE CHICK, by Bradley M. Patten, Western Reserve University, 168 pages, 182 figures, P. Blakiston's Son & Company, Philadelphia, 1920.

Preface. The fact that most courses in vertebrate embryology deal to a greater or lesser extent with the chick seems to warrant the treatment of its development in a book designed primarily for the beginning student. To a student beginning the study of embryology the very abundance of information available in the literature of the subject is confusing and discouraging. He is unable to cull the essentials and fit them together in their proper relationships and is likely to become hopelessly lost in a maze of details. This book was written in an effort to set forth for him in brief and simple form the early embryology of the chick. It does not purport to treat the subject from the comparative view point, nor to be a reference work. If it helps the student to grasp the structure of the embryos, and the sequence and significance of the processes he encounters in his work on the chick, and thereby conserves the time of the instructor for interpretation of the broader principles of embryology it will have served the purpose for which it was written.

THE STORY OF THE AMERICAN RED CROSS IN ITALY, by Charles M. Blakewell, 254 pages, 20 Illustrations, The Macmillan Company, New York, 1920.

Introduction. The purpose of this book is not to give a detailed statistical account of Red Cross activities in Italy,—that may be found in the various Department Reports,—but rather to tell the American people who contributed so generously to the Red Cross funds the simple tale of what their dollars did in Italy. It is a great and inspiring record and one in which Americans may well take pride.

Resumen por el autor, George L. Streeter.

La emigración de la vesícula auditiva del renacuajo.

En el curso ordinario del desarrollo la vesícula auditiva del renacuajo experimenta una emigración definida, moviéndose desde el sitio en el cual se desprende de la piel a una posición más medial y dorsal, de tal modo que eventualmente viene a situarse muy cerca de la superficie lateral del cerebro posterior, con el apéndice endolinfático recubriendo el borde del delgado velo medular que forma el techo del cuarto ventrículo.

Esta particularidad, aparentemente, no es simplemente el resultado de un ajuste producido durante su desarrollo por la interacción de los procesos mecánicos de las estructuras adyacentes, sino que se debe, al menos parcialmente, a una tendencia autostática inherente a la vesícula misma, por medio de la cual mantiene y ajusta exactamente su posición durante al desarrollo con relación al cerebro y las estructuras que le rodean.

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MIGRATION OF THE EAR VESICLE IN THE TADPOLE DURING NORMAL DEVELOPMENT

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ELEVEN FIGURES

In 1837 von Baer made the observation that the diaphragm is situated in the neck region in very young embryos, receiving its innervation from the cervical nerves, and that in the course of its development it acquires a more caudal position corresponding to the enlargement of the heart and lungs. This descent of the diaphragm was subsequently described in greater detail by Mall ('97). Uskow ('83) and Mall ('97) pointed out the marked shifting of position which the heart, lungs, liver, intestinal tract, and Wolffian bodies undergo during development in relation to each other and to the vertebral column. The migration of these organs in the embryo has given us the explanation of the peculiar course of their nerves of supply; for example, the inferior laryngeal, the vagus, the phrenic, and the splanchnic nerves. Kölliker ('61) showed that the shifting in position of the spinal cord produces an elongation of the spinal-nerve roots and the formation of the cauda equina. The influence of this factor in the formation of the filum terminale has been recently studied by Kunitomo ('18). It has been shown by Lewis ('01, '10) that such muscles as the trapezius and the eye muscles undergo considerable shifting in position between the time of their first appearance and the time when they have acquired their permanent attachments. Among others, Futamura ('06) has described the migration of the facial muscles and the consequent deflected distribution of the branches of the facial nerve. Within the central nervous system there are several instances where the

component nuclear masses exhibit a distinct migration in the course of their development (Streeter, '08; Kappers, '10). As a result of their disproportionate growth, the primary divisions of the brain shift into new positions relative to each other, and this is accompanied by an interesting adjustment on the part of the vascular drainage of these structures. Kohn ('07) and others have shown that the sympathetic ganglia undergo an extensive peripheral migration. When the places of origin of the thymus and thyroid glands were first discovered, it was recognized that these organs exhibit a conspicuous type of migration. Even in the case of the skeleton, it has been maintained (Rosenberg, '76) that the point of vertebral articulation of the pelvic girdle moves along the column into the lumbar territory during development, although this has not been adequately substantiated. There is, however, a very good example of topographical adjustment of bony structures in the case of the teeth.

To any one occupied with the study of organogenesis, the developmental alteration in topography that is everywhere in progress is very striking. In some cases it is obviously a matter of mechanical stress exerted by adjacent organs upon each other, the controlling factors being their relative increase in size and the relative resistance of their tissues; or it may be a matter of traction in connected organs. In other instances we find structures invading new territories by virtue of the direction of their growth, which is dependent on the fact that the proliferation and increase in size of their constituent cells are more active in one direction than in another. In some cases this is associated with a thinning out and disappearance (possibly dedifferentiation) of the opposite pole of the organ, resulting in its complete transposition. Such factors are easily understood and various combinations of them explain most of the instances of developmental topographical alteration in organs which we encounter. There are other cases, however, which are more obscure and in which the movement of the organs or structures during their development cannot be entirely explained by simple mechanical factors; in these the phenomenon resembles a true migration such as is seen in individual cells. For lack of a better explanation, we

must consider the possibility of the existence of some force, of the nature of a chemotaxis, interacting between these structures and their environment. It is in this group that we must place the ear vesicle which, during the course of normal development, exhibits a considerable change in position. It is to this that I would call attention here.

From several studies previously reported by the writer (Streeter, '07, '09, '14) and from a recent paper by Ogawa ('21), it is apparent that the determining factors in the posture of the membranous labyrinth involve something more than a passive development of the ear vesicle in the position in which it originally finds itself. It is clearly evident, moreover, that the final position of the labyrinth is not simply the result of an adjustment brought about during its development by the interaction of mechanical processes of the adjacent structures, but that it is due, in part at least, to an autostatic tendency inherent in the vesicle itself, by virtue of which it maintains and accurately adjusts its position during development with reference to the brain and the surrounding structures. When an early ear vesicle is experimentally rotated into an abnormal position, or transplanted in an abnormal position to the opposite side of the same specimen or to another specimen, it subsequently tends to correct its posture, and the final labyrinth, in spite of the previous displacement, is found to possess normal topographical relations.

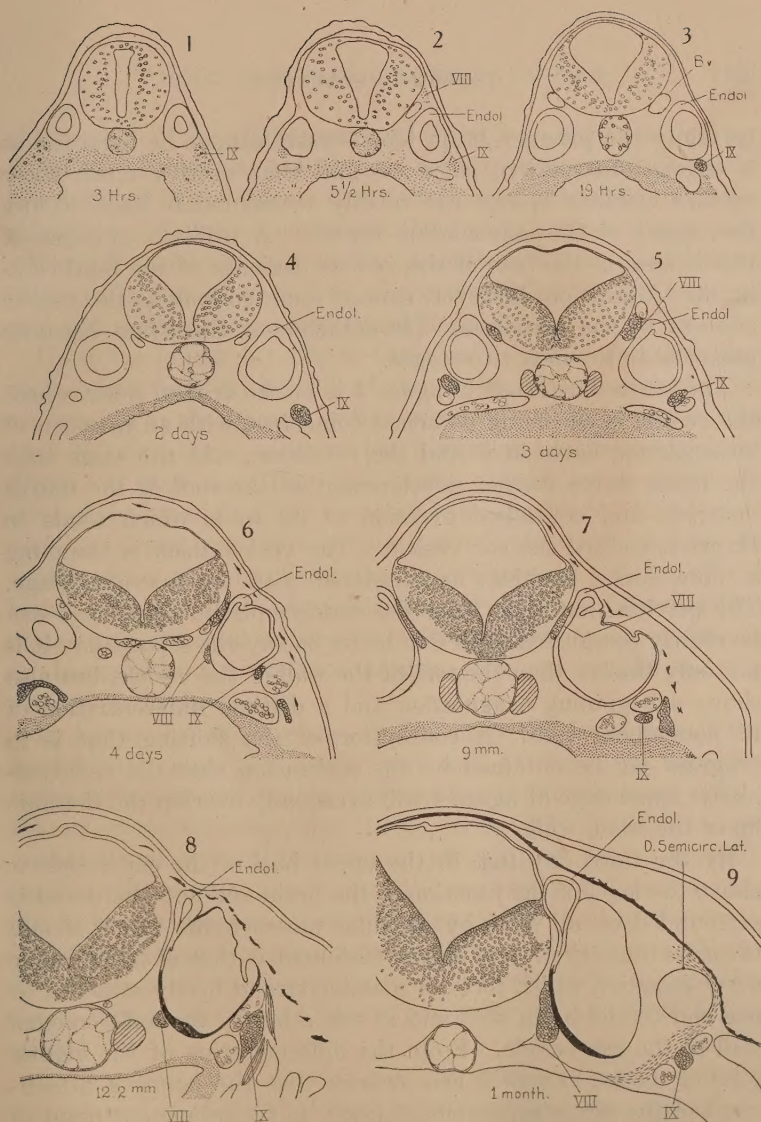
It is of interest to record that not only under artificial conditions, but also in the ordinary course of development, the ear vesicle of the tadpole undergoes a definite migration, moving from the point of its detachment from the skin to a more median and dorsal location, so that it eventually lies close against the side of the hindbrain with its endolymphatic appendage overlapping the margin of the thin medullary velum that forms the roof of the fourth ventricle.

If one prepares sections through the auditory region in a series of tadpoles covering the period between the premotile stage and the end of the first month, the changing relations of the ear vesicle to the surrounding structures can be readily made out (figs. 1 to 9). These figures are made with the same

magnification and thus, by comparing them, it is possible to determine the actual increase in size, as well as the differentiation of the walls and the alteration in the position of the individual vesicles. In figures 7 and 8 the length of the specimens is given; in the remaining figures the age given is the length of time the specimen was allowed to develop after reaching the operating stage, i.e., when it has acquired a distinct tail bud and gill eminences, but has not yet exhibited any motor response to stimuli.

In the first stage shown (fig. 1) the relatively thin lateral wall of the ear vesicle lies tight against the ectoderm. The vesicle is separated from the thick endoderm and the brain tube by a narrow interval filled with mesenchyme which is beginning to show open spaces in the vicinity of the notochord, elsewhere being relatively compact and heavily laden with yolk. As yet there are no blood-vessels in this region and the acoustic nerve and ganglion are not clearly differentiated from the surrounding tissue. It will be noticed that the lateral plate of the brain tube lies in a vertical plane and the point at which the ventral nerve roots are to converge lies opposite the dorsal tip of the ear vesicle.

In the next stage (fig. 2) the ear vesicle remains in close contact with the ectoderm. The surrounding mesenchyme is assuming a reticular character and in it the primary blood-vessels can be recognized. The acoustic ganglion is distinctly marked off, being attached to the anteromedian surface of the vesicle and connected by a strand with the brain wall. The roof of the latter is thinning out and the lateral walls are undergoing eversion. In the third stage (fig. 3) the conditions are essentially the same, although the vesicle wall has undergone further differentiation, the mesenchyme is distinctly reticular, and there is further eversion of the brain wall. In the sections oral to the one selected for illustration, the acoustic-nerve ganglion is present. It can be seen that the ear vesicle at this time is widely separated from the brain and almost wholly ventral to it. The intervening mesenchyme is loose and would offer slight mechanical obstruction to the migration of the vesicle, and



Figs. 1 to 9 Sections showing the changes in the topographical relations of the ear vesicle of the tadpole during the period between the premotile stage and the end of the first month. In figures 1 to 6 and figure 9 the age given is the length of time the larva was allowed to develop after reaching the operating stage; i.e., tail bud and gill eminences present, but no motor response to stimuli exhibited. $\times 50$. B.v., primitive blood-vessel plexus; Endol., endolymphatic appendage; VIII, acoustic nerve ganglion; IX, glossopharyngeal nerve ganglion.

certainly the primary brain blood-vessel cannot be regarded as a serious obstruction, since we find that even a much more mature vascular system can readily accommodate itself to any movement of the surrounding organs. A brilliant example of this is seen in the case of the venous drainage of the fetal cerebrum. Migration, however, cannot occur so long as the vesicle adheres to the ectoderm. Its detachment therefrom becomes complete in the next two stages.

The stage illustrated in figure 4 is at the critical point where the vesicle is becoming detached coincident with an invasion of mesenchyme between it and the ectoderm. At the same time the brain shows further development of the roof of the fourth ventricle and continued eversion of its walls which tends to thrust it toward the ear vesicle. The vesicle itself is assuming a more dorsal position, as compared with the previous stage. The portion that is to form the endolymphatic appendage can be clearly recognized from five hours on; by the second day it is not only thicker than the rest of the wall of the vesicle, but also shows a beginning evagination and a distinct differentiation of its component cells. A conception of the shifting that is in progress can be obtained by the realization that the endolymphatic appendage of figure 4 will eventually overlap the rhombic lip of the brain wall.

By the third day (fig. 5) the upper half of the ear vesicle is above the level of the junction of the brain and notochord and is surrounded on all sides by reticular mesenchyme which should favor its migration. Its only attachment is that of the acoustic nerve ganglion which forms a massive strand firmly attached at one end to the brain wall and at the other to the anteromedial wall of the ear vesicle. From the differentiation of the mantle zone of the brain wall it can be seen that the point of attachment of the nerve corresponds closely to its permanent point of entrance and, tracing it peripherally to the vesicle wall, its fibers can be followed to the macular area. The size and character of the acoustic nerve might lead one to attribute to it a definite influence in any subsequent movement of the vesicle; but we know that the phrenic nerve exhibits no restraining influence in

the descent of the diaphragm and there is no evidence that the facial nerve exercises any guiding force in the migration of the musculature of the face. It is to be noted that there is still a relatively wide interval between the vesicle and the brain wall. As yet the mesenchyme shows no differentiation into skeletal framework, but on each side of the notochord can be seen the oral extension of the spinal musculature.

Up to the fourth day (fig. 6) there has been a gradual thinning of the main part of the wall of the ear vesicle, accompanied by an increase in the amount of the contained otic fluid, and at this time the first steps occur in the formation of the semicircular ducts. A little more than half of the vesicle is now above the level of the junction of the notochord and the brain. The vesicle and brain wall are more closely approximated, which may be explained in part by the further eversion and growth of the latter. On the other hand, there is a beginning differentiation of the subcutaneous tissues and pigment membrane producing an increase in the distance between the ear vesicle and the surface of the larva.

In larvae 9 mm. long (fig. 7) one finds the formation of the semicircular ducts well under way and at the same time the mesenchyme lateral to the ear vesicle is differentiated into a characteristic subcutaneous tissue, while that median to the vesicle shows a condensation into precartilaginous tissue. Spreading from the chordal area toward the ear vesicle and surrounding the brain can be seen arachnoidal spaces of a primitive type. Notwithstanding this more permanent type of environment, the dorsal migration of the vesicle is not yet complete, for in older stages almost the entire vesicle lies dorsal to the level of the chorda.

In larvae 12 mm. long the endolymphatic appendage and the dorsal crest of the vesicle have nearly reached the level of the rhombic lip, and at the same time the lowest point of the vesicle lies opposite the level of the center of the notochord. The dorsal extension at this time is due in part to the direction of the growth, associated with the formation of the anterior and posterior semicircular ducts and the increase in the length of the endolymphatic appendage.

At one month the ear vesicle is completely differentiated into a membranous labyrinth with three semicircular ducts and a characteristic macular area to which is attached the ganglion and its peripheral nerve terminations. A characteristic endolymphatic appendage is present, consisting of a relatively large sac connected by a slender duct with the vestibular portion of the labyrinth. As can be seen in figure 9, the sac now lies in close contact with the thin roof of the fourth ventricle. The labyrinth lies wholly dorsal to the midlevel of the notochord and is secured in this position by the mesenchymal otic capsule,

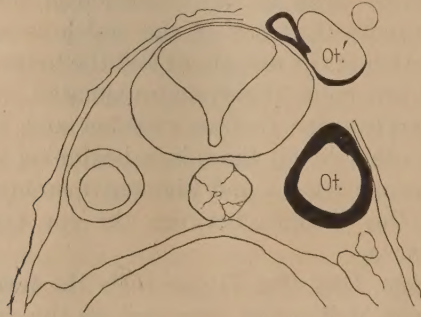


Fig. 10 Diagram showing the migration of the ear vesicle relative to the brain wall from the position it occupies at the end of the second day (*ot.*) to the position it attains as a differentiated labyrinth at the end of the first month (*ot'*), the brain wall being represented as stationary.

consisting of precartilage tissue, portions of which are already differentiated into typical cartilage cells surrounded by a homogeneous matrix. With this stage the essential relations of the labyrinth may be regarded as established; the subsequent minor changes in its topography are those determined by the mechanical factors of its own further growth and the further growth and differentiation of the surrounding structures.

From the foregoing comparison of the individual stages it is clear that the ear vesicle shifts its position relative to the brain wall to the extent diagrammatically shown in figure 10. Whereas at the end of the second day the vesicle lies ventral to and apart

from the brain, at the end of the first month it is situated close against the lateral brain wall with its endolymphatic appendage overlapping the rhombic lip. In the figure there has been no account taken of the lateral movement of the brain wall, and therefore to that extent the path of migration of the ear vesicle is exaggerated. Its dorsal migration relative to the notochord, the ectoderm, and the everted brain wall may be represented as in figure 11, which shows more accurately than figure 10 the extent of its change of position relative to the whole environment. Although the normal migration of the ear vesicle is not so marked

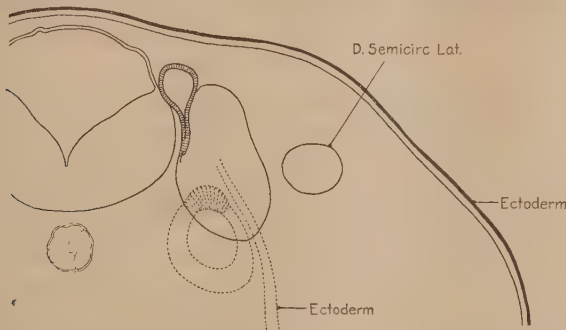


Fig. 11 Superimposed sections of the ear region of a tadpole of the nineteenth hour (dotted) and of another at the end of the first month, enlarged so that the brain is the same size in both cases, the two being fitted so as to exactly coincide.

as that of the thymus and many other organs, nevertheless that phenomenon unquestionably occurs. To some extent the mechanical forces of growth of the concerned parts can be recognized as influencing the migration; no single one of these factors, however, or no combination of them would appear to adequately explain it.

The detachment of the vesicle from the skin is readily explained by the differentiation of the subcutaneous tissues and the formation of the pigment membrane. This begins to take place about the second day. By the fourth day the elements of the pigment membrane make their appearance, and in tadpoles 12 mm. long there is a relatively complete membrane separating the vesicle

from the loose tissue underlying the ectoderm. This differentiation releases the vesicle from its firm attachment to the ectoderm, but it does not in any way favor its dorsal migration.

The change in the relative position of the ear vesicle and the brain wall is in part accounted for by the direction of growth of the latter, which undergoes an eversion whereby it is thrust ventralward and lateralward toward the vesicle. The maximum effect of this eversion is reached at the end of the fourth day, but at this time, in addition to the close approximation of the brain wall and the ear vesicle, a dorsal shifting of the latter has occurred relative to the level of the notochord, a fact which can hardly be explained by the change in position of the lateral brain plate.

As to this dorsal migration of the vesicle as a whole, which takes place gradually throughout the first month, one should consider the possibility of its being due to the direction of growth of the vesicle; i. e., that the dorsal portions of the vesicle may perhaps grow more rapidly than the ventral portions. In the case of the endolymphatic appendage, the direction of growth may very well constitute a factor in the attainment of its final position. The dorsal growth of the sac and the elongation of its duct would favor its dorsal shifting. Aside from the slender endolymphatic duct, however, there appears to be nothing to prevent the sac from occasionally going astray orally or caudally, where the tissues would offer little obstruction to its extension in these directions. That it never does so forces one to postulate the existence of some form of determinative attraction between the endolymphatic sac and the medullary roof to which it later invariably becomes intimately attached.

There is no evidence that the acoustic nerve ganglion plays any considerable part in the way of a guiding or traction force. The nerve can be recognized at the fifth hour, connecting the vesicle with the brain wall, but when it is experimentally detached, as in the transplantation of a vesicle from one tadpole to another, the severing does not interfere with a correct adjustment of the posture of the vesicle. This corresponds to our experience with other organs, in which the nerves do not act as a check or show

any evidence of influencing the migration of the organs in any way. The surrounding mesenchyme and primitive blood vessels can also be dismissed as factors.

The cartilaginous skull does not make its appearance until the final relations of the labyrinth to its environment are essentially established, that is, toward the end of the first month, and therefore cannot play a primary part in the migration of the vesicle. However, after the firm otic capsule becomes differentiated the latter must absolutely control those further alterations in the posture of the contained labyrinth which are associated with the final changes in the form of the base of the skull.

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Resumen por los autores, E. L. y E. R. Clark.

El carácter de los linfáticos en el edema experimental.

Los autores han producido edema en el renacuajo mediante: (1) Extirpación del pronefros; (2) extirpación del esbozo del corazón; (3) impidiendo el desarrollo de la musculatura del corazón linfático, y (4) en conexión con la inflamación aséptica. 1. En los individuos sin pronefros, el líquido plasmático se acumula tan solo en la cavidad abdominal. La circulación sanguínea se hace más difícil y el desarrollo de los vasos sanguíneos se retarda. Los linfáticos se desarrollan, y en apariencia funcionan normalmente. 2. Cuando se extirpa el corazón el edema está limitado a las cavidades del cuerpo. Los capilares linfáticos de la cola aparecen un poco más grandes que lo normal. 3. En renacuajos desprovistos de corazón linfático contráctil grandes cantidades de líquido se acumulan en los senos cefálicos, y eventualmente en el tejido subcutáneo del cuerpo y cola. 4. En el edema inflamatorio los capilares linfáticos de la región afectada se distienden.

En embriones de pollo, los autores impidieron el desarrollo del corazón linfático cortando la cola en los embriones de tres días. A los siete días los embriones están edematosos. La inyección demuestra la presencia de linfáticos distendidos en forma de saco, en vez de aparecer como los conductos más pequeños, presentes en los embriones normales. Conclusiones: 1. Los capilares linfáticos se desarrollan normalmente, absorbiendo linfa, sin circulación sanguínea o con circulación defectuosa. 2. Cuando se impide la salida del líquido que ocupa los linfáticos estos se distienden. 3. La acumulación de fluido en los espacios de los tejidos está asociada con una dilatación de los capilares linfáticos. Este resultado se opone a la idea generalmente mantenida por los patólogos, de que los capilares linfáticos se contraen en el edema.

THE CHARACTER OF THE LYMPHATICS IN EXPERIMENTAL EDEMA

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FIVE FIGURES

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INTRODUCTION

Abnormal collections of fluid in the tissue spaces and serous cavities may be caused by a disturbance in any of the factors concerned with the normal formation transudation and absorption of lymph in the animal organism. The causes are probably different in the different types of edema and a number of causes may be involved simultaneously (Wells, '18). Although the lymphatic system is known to be intimately concerned with the normal absorption of fluid, the relation of the lymphatics to edema has never been thoroughly studied. It is well known that edema of a limb may be caused by blocking of the main lymph channels or lymph glands draining the limb, but this type is of relatively rare occurrence in adult warm-blooded animals, owing to the ability of the veins to take over a large share of the absorptive function of the lymphatics.

Smith and Birmingham ('89) have reported a case of edema of the foetus in which they claim that there was a total absence of lymphatic system.

The reaction of the lymphatic capillaries to the presence of an increased amount of tissue fluid, found in cases of generalized edema of the subcutaneous tissues, is not well understood. According to Adami ('09), the delicate walls of the lymphatic capillaries collapse under the increased pressure of the surrounding fluid.

We have attempted to produce edema experimentally by several methods with the particular object of studying the effect of this condition upon the lymphatic capillaries. Tadpoles were used for these experiments, because of the possibility of watching the lymph capillaries in the living, in the transparent-fin expansion. Some experiments were also performed upon chick embryos.

EXPERIMENTAL EDEMA IN AMPHIBIAN LARVAE

Two species of frog larvae were used—*Rana pipiens* and *Rana catesbiana*. The operations were performed under the binocular microscope, using chloretone anaesthesia. Small glass needles were used for most of the dissections and iridectomy scissors for the removal of the anlage of the blood heart.

The lymphatic vessels of the edematous specimens and of normal larvae of the same ages were injected with india ink. The capillaries of the transparent fins were studied in the living in the observation chamber previously described (E. R. Clark, '12).

The following methods were tried for producing edema in tadpoles: 1) removal of the pronephros; 2) removal of the anlage of the blood heart; 3) removal of somites to prevent the development of the lymph heart; 4) injection of drops of croton oil to produce inflammation (E. R. and E. L. Clark, '20); 5) acetic acid.

1. Removal of the pronephros

This experiment was performed upon a number of tadpoles, during the spring and summer of 1915. Since the general re-

sults of this operation have been described by Howland ('16) and Swingle ('19), it is unnecessary to give a detailed description. Edema developed on the day following the operation and was of the type of ascites, fluid collecting only in the abdominal cavity. During the succeeding days, the development of the tadpoles was greatly retarded, the gills remained external for a longer period than in the controls, the coils of the intestine were fewer, the head and eyes smaller, the tail remained much shorter and more pigmented, and the heart beat sluggishly.

Microscopic observation of the transparent tails of such larvae showed a more sluggish circulation and fewer blood capillaries than in the normal specimens. However, the lymph hearts of the larvae with pronephros removed beat as strongly as did those of the controls, and even more frequently, and the lymphatic vessels of the tail had extended beyond the blood-vessels and were normal in appearance.

2. Removal of the blood heart

The operation for the removal of the heart in tadpoles has been described by Knowler ('07) and the effect upon blood-vessels and lymphatics by E. R. Clark ('18). Embryos operated on in this manner soon become edematous—the collection of fluid being confined to the body cavities, the tail remaining small and pigmented.

Although the development of the blood-vessels is greatly retarded with the heart absent, that of the lymphatics is not. The lymph hearts are larger and beat more strongly than do those of normal larvae of the same age and there is an active movement of fluid inside the lymph-vessels as demonstrated by the occasional presence of blood-cells within the lymphatics, which were observed to move along with the current.

In studying the lymphatic capillaries of the tail it was found that these vessels develop normally and often extend beyond the blood capillaries, although in normal embryos of the same age and species, the blood-vessels of this region grow out well in advance of the lymphatics. Moreover, the lymphatics are wider than in normal animals (E. R. Clark, '18).

This experiment showed that lymph continues to pass into the lymphatic capillaries in the absence of the heartbeat and blood circulation and that the growth and absorptive power of lymphatics are not dependent upon the blood pressure.

3. *Operation to prevent the development of the lymph-heart musculature*

For this experiment the somites dorsoposterior to the pronephros were removed on both sides of the larva. In the majority of cases this effectively prevented the development of the pulsating lymph hearts. Such tadpoles developed normally and were practically indistinguishable from the controls for the first four or five days after the operation. On the sixth or seventh day after the operation, edema of the head region of these embryos makes its appearance. This enlargement was noticed invariably on the same day at which the first pulsation of the lymph hearts was observed in normal tadpoles of the same age. During the following days the sinuses of the head became still more distended, while those at the sides of the body also enlarged and finally, ten to fourteen days after the operation, the tail became edematous. In contrast to the larvae deprived of their pronephroi or blood hearts, these tadpoles did not contain an excessive amount of fluid in their abdominal cavities.

The tadpoles without beating lymph hearts lived for three weeks after the operation—the longest period of survival being twenty-six days. During the first two weeks after the operation the tadpoles were as large as the controls, and practically normal except for the edema. During the last week of life, however, the blood circulation became impaired and often ceased altogether.

In the larvae without lymph hearts, the lymph-vessels were studied by injection of india ink into the main dorsal and ventral lymph-vessels of the tail. The dorsal vessel normally empties into the right lymph heart and the ventral vessel into the left. In the case of the operated tadpoles, the dorsal vessel emptied into a large sinus on the right side and the ventral vessel into a

similar sinus on the left side. These tail vessels together with their branches were found to be wider than those of the controls and the pressure inside them was found to be high, as shown by the effort required to fill them with the injection material.

Microscopic examination of the transparent tails of the tadpoles without lymph hearts showed no difference from the normal during the first week after operation. At the end of ten or twelve days the tail begins to show evidences of edema particularly at the base. During the next week these changes become visible throughout the whole tail. The actual increase in the thickness of the tail was measured by means of the micrometer screw of the fine adjustment, by focusing on a certain point on the surface of the fin and turning the screw until the opposite surface came into focus, and meanwhile counting the divisions on the screw. The edema is further detected by the changed appearance of the tissue in the fin; the whole region becomes clearer and the connective-tissue cells are more widely separated than in the normal tadpoles. The cells themselves do not enlarge.

Associated with this increase in fluid present in the connective-tissue spaces, we invariably found an enlargement of the lymphatic capillaries. The vessels near the base of the tail are first affected and later the more posterior ones enlarge also. Figures 1, 2, and 3 show the difference in size of the lymphatic capillaries in these larvae in contrast to that of the blood capillaries of the same specimens and to that of the lymph capillaries of normal tadpoles.

In addition to change in caliber, lymphatics of these tadpoles with generalized edema also show changes in contour—the irregular outline with abundant fine processes characteristic of the normal lymph capillary is lost and the endothelium becomes smoother and thinner.

4. Inflammatory edema

The cell reactions which take place after injection of minute globules of croton oil into the tail fins of amphibian larvae, have been described in a recent publication (E. R. and E. L. Clark, '20).

In a number of instances a localized edema was observed at the site of injury. The increase in the amount of fluid in the region was easily detected by the increased transparency of the region and by the greater distance between the connective-tissue cells. The increase in the thickness was measured by means of the micrometer fine-adjustment screw. The lymphatic capillary sprouts of the edematous region are always wider than similar vessels of neighboring areas; in fact, the simultaneous enlargement of

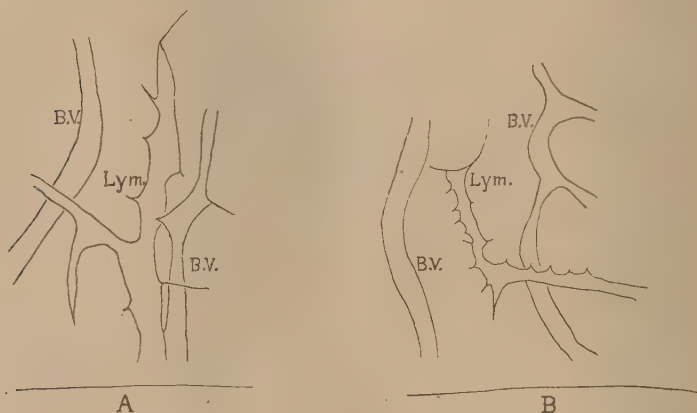


Fig. 1 A. Drawing of vessels from the ventral fin of a tadpole ten days after operation for removal of the lymph hearts. *Lym.*, lymphatic; *B.V.*, blood-vessel. Thickness of the tail at this point, 310μ . B. Drawing of the same region in the ventral fin of a normal tadpole of the same age. Thickness of the tail, 180μ . Note difference in the size of the lymphatic in the two specimens. *B.V.*, blood-vessel; *Lym.*, lymphatic. $\times 175$.

these capillaries was found to coincide precisely with the increase in intercellular fluid (fig. 15, in article by E. R. and E. L. Clark, *The American Journal of Anatomy*, May, 1920). This enlargement of the lymphatics is characterized by a widening of the lumen of the lymphatics, while the endothelial wall becomes thinner and smoother. These lymphatics regain their normal caliber and contour with a return of the region to its normal thickness. The blood capillaries of such regions show no such changes.

5. Experiments with acetic acid

These experiments were suggested by the work of Martin Fischer ('15) which connects the development of edema with the presence of an acidosis in the tissues. Tadpoles were placed in

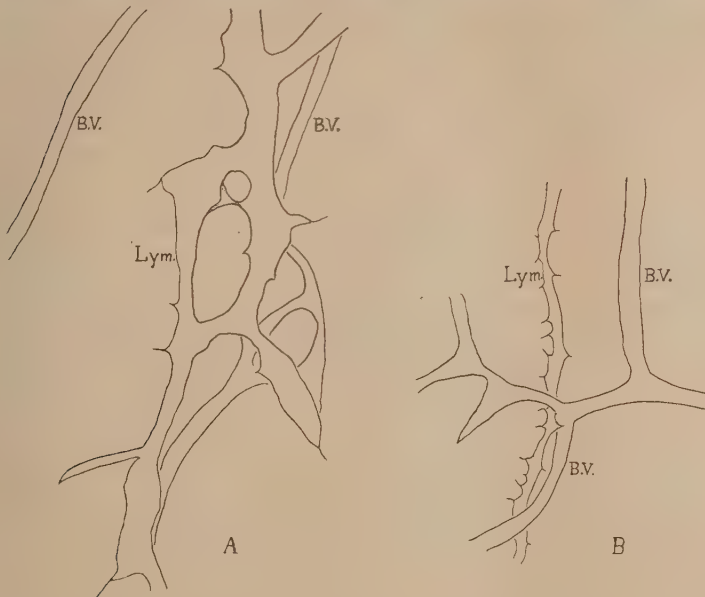


Fig. 2 A. Drawing of vessels from the dorsal fin of a tadpole fourteen days after operation for removal of the lymph hearts. Thickness of the tail at this point, 340 μ . B. Vessels from the same region of a normal tadpole of the same age. Thickness of the tail, 170 μ . This shows still greater enlargement of the lymphatic capillary than in figure 1, A. B.V., blood-vessel; Lym., lymphatic. $\times 175$.

varying strengths of acetic acid, with the object of producing edematous tadpoles. The results of these experiments were as follows:

All tadpoles in strengths of acetic acid of 1 to 2000 or stronger were dead at the end of an hour and a half.

Tadpoles left overnight in strengths of acetic acid from 1 to 5000 up to 1 to 15,000 were all dead on the following morning.

Tadpoles in 1 to 20,000 and 1 to 30,000 survived, and at the end of a week they were as large, active and well developed as the controls and showed no signs of edema.

Tadpoles placed in 1 to 18,000 and 1 to 19,000 died within twenty-four hours.

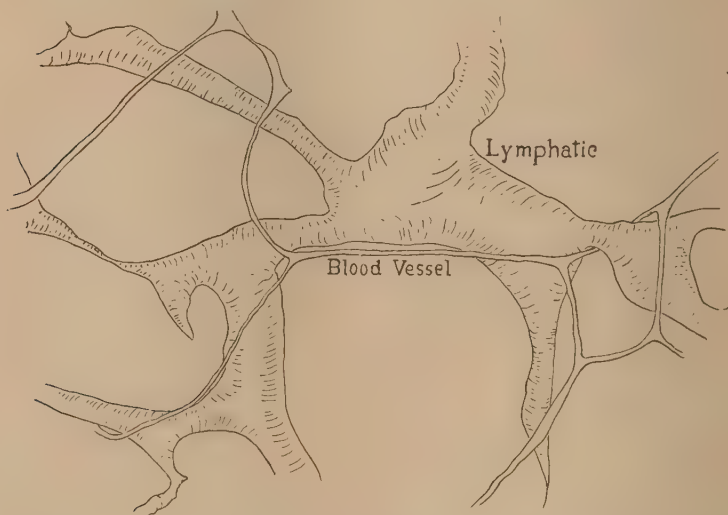


Fig. 3 Drawing of the vessels of the posterior portion of the abdominal wall of an edematous tadpole fifteen days after operation for the removal of the lymph hearts. The lymphatics are greatly distended while the blood-vessels are narrower than normal. $\times 158$.

These experiments were negative in regard to the production of edema in tadpoles by the use of acetic acid in the surrounding fluid, since all specimens died when the acid was stronger than 1 to 20,000, while in this and in weaker strengths of acid no edema developed. Obviously, these results do not necessarily have any bearing whatever on the theory that edema may be caused by acidosis, since the presence of a trace of acid in the surrounding medium may have had no effect on the acidity of the body fluids.

EXPERIMENTAL EDEMA IN CHICK EMBRYOS

We attempted unsuccessfully to produce edematous chick embryos by chemical means—acetic acid was placed in an open dish in the incubator and alcohol also was tried without success. Only one form of edema was produced in chick embryos—that which resulted from the removal of the posterior lymph hearts. The development of the lymph heart is prevented by cutting off the tail rudiment in embryos of two to three days' incubation. This operation and the early development of the lymphatics in such embryos have been described elsewhere (E. R. and E. L. Clark, '19).

It was shown in a former publication (E. L. Clark, '15) that the lymph flow in the early superficial lymphatics of chick embryos is dependent to a considerable extent upon the pulsation of the posterior lymph hearts. It was shown that the commencement of lymph heart pulsations, in chicks of $6\frac{1}{2}$ days, is the factor which instigates the lymph flow in the posterior half of the embryo. The area drained by the lymph heart increases until, in embryos of seven to eight days, the direction of the entire superficial lymph flow is posterior through the lymph hearts into the veins of the tail. Associated with the establishment of the lymph flow in the superficial lymphatic plexus, channels develop in the exact places where the movement of lymph had been demonstrated in the living chick by the injection of india ink into the superficial lymph capillaries. In addition to the formation of these ducts, the former investigation showed that the lymphatics in later stages enlarge to form sacs at points where two conflicting pressures occur. In this later stage, eight to nine days, the tissue is very loose—so much so, in fact, that it might almost be called edematous, were it not for the fact of its normal occurrence. At this later stage the lymph hearts are chiefly concerned with the flow of lymph from the allantois.

In the former publication a case was described in which an embryo of seven days possessed a stunted tail with a small feebly beating lymph heart. This embryo was edematous and the lymphatics of the pelvis which drained into the lymph heart were

large and distended, greatly resembling the sacs normally present in this region in chicks of eight and one-half days.

In the operated tailless chicks of six and one-half days, the stage at which the lymph heart begins to beat in normal chicks, the anterior lymphatics are normal as regards appearance, development of the main duct, and direction of lymph flow. The posterior half of the body, however, is markedly edematous in these chicks and the channels normally present over the pelvis



Fig. 4 Drawing of an embryo chick of seven days, in which the tail containing rudiment of the lymph hearts had been removed at the three-day stage. Compare character of the lymphatic plexus, injected with india ink, with that of a normal chick of the same age (fig. 5). The absence of definite channels in the posterior half of the body and the enlargement of the lymphatic capillaries over the pelvis are particularly noticeable. $\times 4.67$.

are absent and in their place is an irregular plexus of the primitive type, the vessels composing which are much larger than usual.

In chicks of seven to seven and one-half days, the stage at which the lymph flow of the entire superficial lymphatic system is normally influenced by the beating of the lymph heart, the chicks were always found to be edematous after the operation for the removal of the lymph heart. The edema is noticeable to the naked eye; it is evident from the greater distance necessary to plunge the injecting cannula before reaching the superficial lymphatics, and also from the greater spaces present between the connective-tissue cells in microscopic sections of such embryos.

In these chicks, channels over the anterior body wall and pelvis are absent and in their place an irregular plexus is present, many of the vessels of which are greatly enlarged and sac-like in appearance (fig. 4). It is evident from the difficulty encountered in



Fig. 5 Drawing of a normal chick of six days and twenty-two hours, showing injected superficial lymphatics with newly formed lymph ducts and lymph heart (L.H.). $\times 5$. (Copied from Clark, E. L., '15, fig. 4.)

injecting these vessels over the pelvis that the fluid in them is under high pressure.

In older stages, that is, in chicks of eight and one-half days and older, the tailless embryos are not noticeably more edematous than normal specimens of the same age. Injections of the superficial lymphatics show in most cases that the lymphatics from the two sides have anastomosed over the stump. Otherwise they

possess the same channels and superficial sacs as the normal specimens. It will be remembered that in normal chicks of eight and one-half days and over, the lymph heart is only slightly concerned with the lymph flow in the superficial lymphatics, being chiefly concerned with the flow of lymph from the allantois. It is interesting to find in these operated chicks of eight to nine days that the lymphatics of the allantois are so distended as to render them easily visible to the naked eye without injection.

These operations on chicks have added more evidence to that already reported with regard to the importance of the lymph heart in instigating and influencing the early flow of lymph in the superficial lymphatic plexus and in determining the formation of ducts in the posterior part of the body. They also show that when the lymph flow is interfered with in these early stages, by removal of the lymph heart, embryos become edematous, the development of ducts is interfered with, while the vessels of the superficial lymphatic plexus enlarge greatly until they become even larger and more distended than the sacs of older chicks.

DISCUSSION AND CONCLUSION

The ease with which experimental edema is produced in amphibian larvae by any interference in the flow of fluid in the lymphatics is undoubtedly due to the importance of the lymphatic system of the lower vertebrates in connection with the absorption of water. Maxwell ('13) has found that a relatively enormous quantity of water passes through the frog's skin, and Moore ('15) has shown that a large part of this absorbed water is carried off by the lymphatics. In the lower vertebrates in which there is an active absorption of water through the skin, lymph hearts which assist in maintaining the flow of lymph are always present. In birds, the two lymph hearts, one on each side of the tail, function during embryonic life, but usually atrophy at the time of hatching (exceptions to this rule being water birds and the ostrich and cassowary). In the higher vertebrates—mammals and most adult birds, in which the absorption of water through the skin is practically absent—the importance of the lymphatic system as a drainage system is evidently diminished. Mam-

mals do not possess lymph hearts, but with the appearance of lymph glands the lymphatic system takes on new and important functions not found in lower vertebrates.

The fact that lymph hearts are present in all birds during development, which takes place in a fluid medium, suggests the possibility that there may be an absorption of fluid through the skin of the higher vertebrates during their embryonic life with a resultant increase in absorption of fluid by the lymphatics. In a former paper (E. L. Clark, '15) it was shown that in the chick embryo lymph sacs develop at a stage in which the pressure inside the lymphatic vessels is high, probably owing to increased absorption and the outlet into the veins interfered with, and that such sacs always develop at points where conflicting pressures occur. It is possible that the early development of lymph sacs in mammalian embryos at points where the lymphatic system communicates with the veins may be due to the fact that this actively functioning drainage system has no pulsating lymph heart to assist in the flow of fluid from the tissues.

Moreover, the present experiments, which have shown the important effects upon the absorption of fluid from the tissues resulting from an interference with the drainage of fluid by the lymphatics, in tadpoles and in chick embryos, suggest the possibility that edema of the human embryo may also be caused by a blockage of the main lymph channels, especially of the thoracic duct.¹

¹ Smith and Birmingham ('89) assert that in the edematous foetus which they studied the lymphatic system was entirely absent. However, the illustration which they give (a low-power drawing of a microscopic section) contains certain structures which are unquestionably lymphatics, and not 'spaces' as they are labeled. Microscopic studies of edematous tissues (Marchand, '11; E. R. Clark, '16, and this article) have shown that fluid in subcutaneous tissues does not collect in 'lakelets,' but that it is evenly distributed, separating the cells in a uniform manner. The more probable explanation for this interesting case would appear to be that instead of being absent, the lymphatic system was blocked at some important point—perhaps at the outlet of the thoracic duct—and that there had then occurred a generalized edema of the embryo and a simultaneous distention of the lymphatics including the finest capillaries. It is interesting to see that in this case the 'spaces' in the mesenchyme are much larger than the blood capillaries.

The experiments reported here have yielded the following results in regard to the relation of the lymphatics to experimental edema:

With the removal of the pronephros or the blood heart in amphibian larvae and the consequent abnormal collection of fluid in the serous cavities, the lymphatics continue to develop and to function in a normal manner. Their development and function are not interfered with even by the absence of circulation in the blood vascular system.

Generalized edema of the embryo may readily be produced by mechanical interference with the outflow of fluid from the lymphatics (removal of the lymph hearts in tadpoles and in chick embryos).

In such cases of generalized edema the lymphatics invariably enlarge and the delicate lymph capillaries do not collapse with the increased pressure outside the vessels, but, on the contrary, they continue to absorb fluid until they become greatly distended.

In cases of localized edema, in areas where an inflammation has been produced, the lymphatic capillaries of the edematous region respond to an increase in the fluid outside by enlarging at the tips, and they resume their normal size with the disappearance of the edema.

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Resumen por el autor, S. E. Whitnall.

Algunos músculos anormales de la órbita.

El autor describe un músculo elevador de los párpados superior que presenta dos anomalías comunes de los fascículos que pasan: (a) A la polea del músculo oblicuo superior (músculo tensor de la troclea, de Budge), y (b) A la glándula lacrimal; y en adición, (c) existe una banda transversa y entrecruzada parcialmente (músculo orbitario transverso). El origen del músculo oblicuo inferior del globo ocular fué examinado en cien órbitas, hallando el autor un desplazamiento lateral de su posición descrita como normal, (inmediatamente adyacente al borde de la incisura lacrimal u orificio superior del canal naso-lacrimal) en muchos casos, y en el 14 por ciento de los casos estaba separado un cuarto de pulgada, próximamente. En un caso, cuando la distancia era 7 mm. la inserción ocular fué examinada, hallando el autor que estaba situada más arriba de lo normal. Otras anormalidades observadas son las de los músculos rectos, mencionadas en la literatura, junto con ejemplos de conexiones entre los músculos, encontradas por el autor en una serie de disecciones.

Translation by José F. Nonidez
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SOME ABNORMAL MUSCLES OF THE ORBIT

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TWO FIGURES

The following instances of abnormal muscles were met within a series of dissections of the orbit.

LEVATOR PALPEBRAE SUPERIORIS

There are three variations from the normal in this instance which was found in the left orbit of an adult female cadaver, of which the other orbit was not available for dissection.

1. The most striking feature is the presence of a band of muscle fibers, 2 mm. broad, passing transversely across the anterior part of the orbit and interwoven to a certain extent with the fibers of the levator palpebrae superioris. The extremities of the band curve slightly backward to be attached to the periosteum of the medial and lateral walls at about the junction of their anterior and middle thirds.

2. From the medial margin of the levator itself a well-defined slip is separated off and passes in the direction of the trochlea or pulley of the tendon of the superior oblique muscle. This slip loses its fleshy character before it reaches the trochlea, being continued on by connective-tissue strands.

3. From the lateral margin of the levator a well-marked offshoot passes both to the orbital wall and also to the lacrimal gland. In two other cases I have seen similar fasciculi passing to the lacrimal gland. They are attached to the connective tissue which forms the so-called capsule at the posteromedial region of the gland, and traction upon the levator draws the gland slightly backward. Microscopical examination showed all these fibers to be cross-striped.

There have been described two abnormalities of the levator of the upper eyelid:

a. Where the muscle presents an offshoot from its medial border which passes to the pulley of the superior oblique muscle, replacing or reinforcing the normal fascial expansion of its sheath to that point. This muscular slip is the *tensor trochleae* of Budge ('59), and is identical, according to Macalister, with muscles described by Vesalius, Molinetti, Kolmus, Sandifort, and with

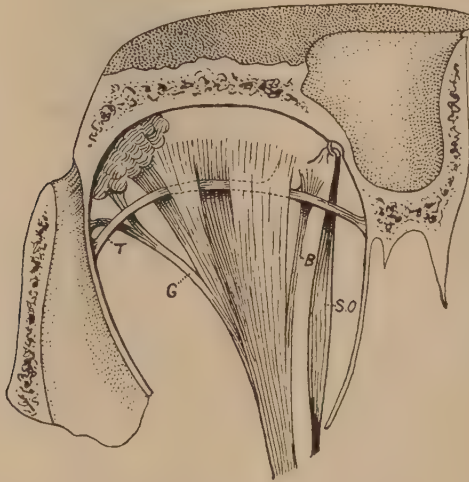


Fig. 1 Left orbit dissected from above to show an abnormal levator palpebrae superioris, showing *T.*, a transversus orbitis; *B.*, a gracillimus or tensor trochlear or comes obliqui superioris, as it is variously termed; *G.*, a slip passing to the lacrimal gland; *S.O.*, is the superior oblique muscle.

the *comes oblique superioris* of Albinus, and *gracillimus orbitis* of Bochdalek ('68). According to Budge, it is found in 10 per cent of cases, but in the writer's experience is much more rarely found in a well-defined state. In one preparation there were present two long muscle bundles, arising in common with the levator and ending anteriorly, one upon the fascia bulbi between the superior oblique and the globe, the other on the orbital margin beneath the pulley; the nerve supply came from the fourth nerve; the superior oblique was broader than usual. Ledouble

has found supernumerary fasciculi accompanying the reflected tendon of the superior oblique, and has further recorded a case where the direct or fleshy part of this muscle was absent, the reflected or normally tendinous part being muscular and arising from the site of the pulley, recalling the type found in non-mammalian vertebrates.

b. The *musculus transversus orbitis*, described by Bochdalek in 1868 as a muscle passing from the anterior and upper part of the os planum of the ethmoidal bone across the upper part of the orbit to its lateral wall. It consists at its origin of small tendinous bands enlarging to fleshy bundles which give off various attachments to neighboring fascia and especially to the levator palpebrae superioris with which it is closely connected; in fact, when the transversus orbitis is small it practically forms part of the levator. Macalister and Ledouble consider it as being a backwardly displaced slip of the orbicularis palpebrarum. This transversus orbitis is sometimes confused with the gracillimus, as, for example, by Howe ('07).

Perna ('05) described an 'abnormal transverse muscle' of the orbital cavity in man, which appears to be a transversus orbitis. He differs from Macalister as to the real origin of the muscle, considering it to represent the remains of the primitive muscular membrane which surrounds the organ of vision in lower vertebrates and which persists longest in phylogeny where the bony orbit is least complete and of least protection; he considers the levator itself to have the same origin, differentiated later for the special function of palpebral movement. An objection to this view is that the orbital periosteum, the periorbita, with its vestige of involuntary musculature found in the infra-orbital fissure in man and much more largely developed in certain lower animals, may be considered on sounder morphological grounds to be the representative of this primitive orbital cavity (Motais, '87; Groyer, '03). It may also be pointed out that the frontal nerve is lying between the planes represented by these two structures—the levator and the periorbita.

Neither would this anomaly appear to be derived from the peribulbar involuntary musculature described by Landström

('07) and recently re-investigated by Hesser ('13), since the fibers are of a different nature and lie on a much more superficial plane.

As regards the view of Macalister that the abnormality is formed by a portion of the orbicularis oculi, it is difficult to see how a portion of the superficial facial muscle sheet comes to be displaced to so deep a plane, posterior to the tarsal plates and septum orbitale, the latter of which is generally regarded as forming the anterior boundary of the orbital cavity, and why such fasciculi are so intimately connected with the levator.

A third explanation which might be considered is that the transversely disposed muscle fibers replace that thickened part of the superficial fascial sheath of the levator which runs in the same direction across the orbit and presents somewhat similar attachments and, it has been suggested, may act as a check ligament to the action of the muscle (Whitnall, '10). This view is supported by the fact that in the present instance the fascial sheath was but little developed and formed no transverse band such as is normally present. The difficulty here is to reconcile the fact that while it is not uncommon to find the reappearance of muscle fibers in connective tissue which has replaced muscle, as, for example, in the case of the panniculus carnosus, yet pre-existing connective tissue tends under strain rather to become condensed and developed into a definite ligament than to be replaced by muscle, as instanced by the appearance of definite ligaments in certain portions of the capsules of joints. On these grounds Perna's view is borne out by the numerous offshoots of the levator which may appear—it may be the remains of a much larger muscular sheet, but it is not homologous with the primitive membranous orbit as represented by the periorbita.

The present instance of an abnormal levator palpebrae superioris muscle is of interest in that it shows, though perhaps feebly developed, the two variations commonly described, the *tensor trochlea* and the *transversus orbitis*, and in addition an offshoot passing to the lacrimal gland. The latter could have a much more effective action as a *retractor glandulae lacrimalis* than the medial offshoots as a *tensor trochleae*, since the gland is fairly movable, the trochlea only slightly so.

MUSCULUS OBLIQUUS INFERIOR

The inferior oblique muscle of the eyeball is stated to arise from the anteromedial part of the floor of the orbit, just within the margin and immediately adjacent to the opening of the nasolacrimal canal (*incisura lacrimalis*). The site may be marked by a small oval impression and often the margin of the orbit is slightly lipped in this region. So close is the origin to the edge of the canal that occasionally fibers of the muscle are found to spring from the periosteal covering (*lacrimal fascia*) of the lacrimal sac at its base (fig. 2).

Since several cases were found by the writer where the origin was displaced laterally some distance from this normal site, it appeared worth while examining a series of orbits. Out of 100 orbits dissected the distance of the origin from the edge of the nasolacrimal canal was as follows:

Adjacent (0 to 1 mm. away).....	in 45 cases
2 mm. distant.....	in 14 cases
3 mm. distant.....	in 19 cases
4 mm. distant.....	in 8 cases
5 mm. distant.....	in 6 cases
6 mm. distant.....	in 4 cases
7 mm. distant.....	in 4 cases
Total.....	100

Approximately, therefore, the origin lay a quarter of an inch (5 to 7 mm.) lateral to the normal site in 14 per cent. the displacement was more commonly found in left orbits.

In both orbits of one subject (female) the origin of the muscle was found situated 7 mm. away from the orifice of the canal, and the insertion of the muscle onto the right eyeball was specially examined (fig. 3). The breadth of the insertion was 8 mm. (usually 9.9 mm.) and was placed above, instead of below, the horizontal meridian; its nearest point was 5 mm. from the optic nerve—a little less than usual; its central point was 9 mm. (usually 11 mm.) distant from a corresponding point on the line of insertion of the superior oblique muscle. The total length of the muscle was 37 mm., which appears normal. In two other cases

of similar origins examined the most marked differences of insertion were again in a higher position on the eyeball, though it should be added that, according to Howe, the ocular insertions of the inferior oblique are more variable than any of the other muscles. The length of the muscles was normal.

As regards the possible effect of such a laterally displaced origin on the action of the muscle, it is first to be noted that, since



Fig. 2 Diagram to show normal position of origin of the inferior oblique muscle and its insertion onto the eyeball.

the attachment is behind the equator of the globe, contraction of the muscle will tend to draw the latter forward, and the more directly the origin is situated in front of the eyeball, at a greater advantage can the muscle so act. In the second place, the effect of the displacement is to bring the origin nearer the insertion, and so the length of the muscle would be decreased. Since the length of a muscle, in which the fibers are arranged parallel to the long axis, is one of the factors which determine the extent

of movement of its insertion, this shortening would tend to influence the range of action. In these cases, however, the disadvantageous position of the origin is compensated by the higher position of the insertion on the globe and the length of the muscle is not affected. The independent action of the normal inferior oblique is to elevate and abduct the cornea and cause the globe to revolve outward on its anteroposterior axis. The first and last



Fig. 3 Diagram to show an instance of abnormal origin of the inferior oblique muscle and its higher attachment onto the eyeball.

of these movements are not impaired by the higher position of the insertion; abduction is chiefly effected by the lateral rectus, though shared by the superior rectus, so that the effect on this movement is negligible.

The superior oblique muscles were examined in the same subjects, especially as in the first case the pulley for the muscle was situated further forward than usual, being almost on the orbital margin (though the position was normal in the other cases exam-

ined). Here the angle between the belly of the muscle and its reflected tendon was apparently less than usual, but accurate measurement was impossible, owing to the nobility of the globe in the advanced stage of the dissection. The angle was certainly much less than the normal 54° , and appeared about 31° . The insertion onto the globe in each of two cases was broader than usual (14 mm. and 11 mm., instead of an average width of 9.5 mm.), but the position as regards the vertical meridian was normal and not of the myopic type (i. e., parallel to and wholly on the lateral side of the meridian); as in the case of the inferior oblique, however, there is much variation in the 'normal' insertion.

From comparative anatomy it is seen that this condition resembles that found in certain fishes, where the oblique muscles arise from the orbital margin more anteriorly, in front of the globe.

RECTI MUSCLES

As regards abnormalities of the recti muscles, it is probable, to judge from the writer's individual experience in finding quite a number of gross anomalies in a series of dissections, that such are by no means as excessively rare as would appear from the number recorded in the literature; in the ordinary dissecting-room conditions do not favor their identification, and in life some may be unrecognizable through compensatory action of the other muscles. They can of course be explained by errors in development by cleavage from the common premuscular mesoblastic mass. In the following notes, mention will be made of such abnormalities as have been recorded in addition to those found by the writer.

The superior rectus has been found to give off a muscular slip 15 mm. long, which arose from the same origin from the annulus of Zinn and passed downward and forward across the lateral face of the optic nerve to join the inferior rectus about its midpoint; the nerve supply came from the inferior division of the third nerve (Aubaret, '09).

The medial rectus has been found absent in some cases of divergent strabismus (Ledouble, '97; Krause). Its posterior third

may be fused with the inferior rectus. A bifid sclerotic insertion by two tendons, 16 mm. in length, is recorded (Wicherkiewicz, '07).

The lateral rectus has likewise been found undeveloped in some cases of convergent strabismus (Ledouble, Krause), and a case of atrophy of the muscle has been noted on operating for strabismus in the living (Bourgeois). A fasciculus may pass from it to the inferior rectus, as is normal in certain ruminants, or to the lateral wall of the orbit (Moseley, '53). A lateral rectus with two extra fasciculi which passed forward to end on the inferior tarsal plate and lateral wall is recorded (Curnow, '73). In a specimen dissected by the writer there was a well-marked, fleshy bundle 7 mm. long and 2 mm. in diameter, passing from the lateral rectus across the posterior third of the orbit beneath the optic nerve to fuse with the belly of the medial rectus; no nerve could be traced to it.

The inferior rectus has been found by the writer to give off a large muscular bundle which passed lateral to the optic nerve and joined the superior rectus; it was innervated by the lower division of the third nerve.

An abnormal muscle bundle (*musculus obliquus accessorius inferior*) has been found by Rex ('87) passing from the apex of the orbit to the inferior oblique, but also sending a slip to join the inferior rectus; it was found in both orbits, and was supplied by the third nerve.

As regards the vestiges of the *musculus retractor bulbi* which have been found in man, the reader should refer to an article by the writer ('11); to the literature therein cited may be added a paper by Hopkins ('16).

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Resumen por el autor, H. E. Radasch.

La determinación del tanto por ciento de substancia orgánica en el hueso.

El tanto por ciento de substancia orgánica en el hueso compacto es 32 a 33 por ciento, según los autores. De la inspección de la literatura general no se desprende el método empleado en la determinación de dicha substancia orgánica. Con el objeto de determinar el tanto por ciento real y hallar, a ser posible, el método empleado por los primeros observadores, el autor ha llevado a cabo varios experimentos de diversa naturaleza. Después de preparar cuidadosamente trozos del fémur, tibia y fíbula, pesó una serie de trozos, que se calcinaron después, pesando el producto de esta operación. La pérdida de peso indica la cantidad de substancia orgánica incinerable que forma parte del hueso compacto.

Entre los veinte a sesenta años de edad, el tanto por ciento medio hallado es 40.75. En el gato adulto el tanto por ciento de peso en del hueso joven es 38.32, mientras que en el conejo (dos tercios del crecimiento total) el tanto por ciento medio es 38.90. Mediante otros métodos, la humedad y las substancias solubles en el alcohol y el éter fueron eliminadas, fijando la cantidad de contenido orgánico fijo. La cantidad media de humedad durante el periodo comprendido entre los veinte y sesenta años es 8.42%, y la relación 7 de la substancia orgánica fija y el hueso seco es solamente 34.92%. La cantidad media de substancia soluble en el éter es 9.27 por ciento; la relación media de la substancia orgánica fija y el hueso extractable es 31.34%. Parece sin embargo que el peso tipo debe ser el del hueso joven, y si se acepta esto, la cantidad media, de substancia orgánica contenida en él es 40.75 por ciento.

THE DETERMINATION OF THE PERCENTAGE OF THE ORGANIC CONTENT OF COMPACT BONE

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In considering the chemical composition of bone we are told that it consists of two main substances intimately commingled, viz., earthy and animal substances. The former comprises the following substances, according to Cunningham's Anatomy ('14):

	<i>per cent</i>
Calcium phosphate	53.23
Calcium carbonate	7.32
Calcium fluoride	1.41
Magnesium phosphate	1.32
Sodium chlorid	0.69
	68.97
Organic material	31.03
	100.00

The organic substances comprise fats and ossein.

According to Piersol, who quotes Berzelius, the inorganic material comprises 67.3 per cent, while the organic material consisting of gelatin and blood-vessels constitutes 33.3 per cent. Schaefer, Prudden (Ref. Handbook of Medical Sciences), Sobotta, all give the same as Piersol, apparently having accepted the same source as their guide. Gray's Anatomy ('18) gives the organic content as from 67 to 68 per cent and so the inorganic constitutes 33 to 32 per cent.

We are further told by Schaefer that the animal material, improperly called cartilage of bone, differs from cartilage physically and chemically. It is much more flexible and softer and upon boiling the bone yields mainly gelatin. He concludes that

the animal material closely resembles white fibrous and areolar tissues in that it consists mainly of collagen.

Normal bone is hard, rigid (to a certain extent), tenacious, and also elastic. The earthy materials contribute to its hardness and rigidity, while the organic material gives bone its tenacity and elasticity. With the last characters in mind, we can readily understand some of the results of fractures. Although we are not told so, we naturally conclude that the foregoing percentages and characteristics apply to compact bone and to the adult type. This being agreed, we naturally would consider that the bones of the young and adolescent would contain a greater percentage of organic material and, therefore, a lower percentage of inorganic substance. This chemical difference, therefore, makes a physical difference to the effect that the bones of the young should, theoretically, be more elastic and tend less to fracture under the same proportionate strain than that of the adult; that the writer believes the surgeon will admit. In the case of ultimate fracture, however, we might expect a different result than in the adult, and consequently we find the green-stick fracture pertains to youth entirely and does not occur in the adult. This is due to the higher percentage of organic substance in the bones of the young. This will be shown actually in the succeeding data.

We are told that by subjecting the bone to an open fire (calcining) the organic substance is burned out, leaving a white, brittle, chalk-like substance that preserves its original shape, but with the loss of about one-third of its weight. This porous cast is easily broken, so apparently the substance which gives tenacity has been removed. We might naturally infer from this that in old age there is a reduction of organic substance, for it is known that in old individuals fractures occur more easily than in those in the prime of life; also repair is less rapid and less satisfactory in old age. We would believe, then, in the elderly, that there is a reduction of organic substance that causes the bone to yield more quickly to strains. Yet Rusby (Ref. Handbook of the Medical Sciences) tells us that as age advances there is a diminution in the mineral constituent of bone and the

organic element is slightly increased. This should make the bone somewhat more tenacious and less prone to fracture—just the reverse of actual experience. He states that this increase is due to the replacement of bone by enlarged blood-vessels and marrow, so that there is, in reality, a reduction of fixed organic material. Yet in those bones examined in this work no reduction was noted, but rather an increase, so that theoretically the bone should not be weakened in old age as it naturally is. There is apparently a thinning or diminution of the actual bony layer of the long bone, and this accounts partly for the readiness of the fracture.

Hoppe-Seyler is quoted (Ref. Handbook of the Medical Sciences) as giving the following composition of dried bone without the removal of the marrow or blood. Water, 50 per cent; fat, 15.75 per cent; ossein, 12.40 per cent; bone earths, 21.85 per cent. Why the bone-marrow and blood should be considered and included in the determination of the organic composition of bone seems strange. These two substances are not a direct part of the bone and they should be got rid of entirely in the determination of the organic constituency. Again, these substances will give some ash and will contain some elements that are concerned in bone formation, so that the effect of calcination of fresh bone with its blood and marrow would be to add to the inorganic constituents the amount of ash of the organic parts. As a result, the percentage of inorganic would be somewhat higher than it should.

For the same reason there is no way in which the organic structure of cancellous bone can be determined, as the marrow contained cannot be got rid of in any way that would not tend to remove some of the soluble organic constituent of the bone. The percentage of organic material would be quite high in such a case.

It would seem that to get the real percentage of organic substance of compact bone, it would be necessary to remove as thoroughly as possible all traces of fat, marrow, and blood-vessels and external, or surface moisture. In that way the organic material remaining would practically belong to the bone and be a part thereof.

Primarily, the reason for the following determination was to find out, if possible, a definite relation, or variation, of the organic constituents of bone at the various ages. Just what methods of procedure were used by those who made the early determinations was not known, so some fresh bone was cleaned, weighed, calcined, and the contents determined. By this method the percentage of inherent, fixed, or component organic substance was so far above that given in the text-books that it caused surprise. There was no reason to believe that it could be an accidental variation, so that the writer immediately wondered how Berzelius, et al., made their determinations. If, according to Hoppe-Seyler, the marrow and blood-vessels were included with the bone, then we can see readily that there would be a variation, but this would make the organic content still higher than the author's determination.

In order to attempt to find out the method used by the older chemists, the determination of the organic content was carried on in a number of different ways.

So as to try to reach as near as possible the true percentage of organic substance in compact bone, five different sets of determinations were carried out. Four of these were applied to every sample of green bone and the fifth applied to the bones used for study by the students. The determinations were made on the bones of adults, varying the ages as much as possible, and stillbirths and even fetuses. In addition, in order to have a comparative anatomy relationship, determinations were also made in rabbits and cats.

The same method of preparation was used in all, except the bones used by the students for study. Certain bones only were used in all ages and in all of the animals, viz., femur, tibia, and fibula. In order to get as fair a sample of compact bone, i.e., where it would be most compact, the middle (from end to end) of each was chosen, and a section cut out and handled in a certain routine manner in all instances. The material was chosen chiefly from postmortem subjects, and not from the remains of the subjects from the dissecting-room for several reasons: 1) To avoid involving the chemicals of the embalming fluid. 2) In

order to determine the normal inherent moisture of the bone as just removed from the body. Determinations were also made in bodies that had been refrigerated for some time, and a difference was noted in the results here also.

After removal of a section of the femur, tibia, and fibula from the same sources, the flesh was allowed to remain on the others until one had been prepared for all four methods, as follows: First, the flesh of one piece of bone is all carefully cleaned off and the periosteum completely stripped, say off the femur. This caused no trouble except along the *linea aspera* of the femur and at all of the borders of the tibia and the fibula. Here the membrane adheres most tenaciously, as many processes extend into the bones at these lines and serve to anchor the membrane firmly in position. Here care must be exercised to get out all traces of Sharpey's fibers, else they might naturally add their mite to the organic constituent of compact bone, and erroneously so.

The next step is to strip out all of the marrow and then cut out all of the cancellous spicules along the inner surface of the bones. In cutting these away considerable fatty matter is thus exposed and removed, thereby getting rid of another error-producing element. When as much of this cancellous bone as possible has been removed, one can feel reasonably sure that the remainder is a real sample of compact bone. Then the bone is carefully wiped externally and internally and a section (ring) about 1 cm. in height is cut; this is then cut into quarters. Each quarter is then carefully wiped again, especially the narrow-cavity side, in order to remove all superficial moisture and fat; it is then weighed and this is the green weight. This is done with all four pieces, so that this green weight serves as an excellent check in the three other determinations.

Next the tibia is treated in the same way and then the fibula. The reason why all are not cleaned at once and then cut and weighed is because, if cleaned and left exposed to the room air, some of the moisture would escape and cause a variation in the determination. For that reason each specimen is treated in this routine manner. This may seem far-fetched, but it is of

the greatest importance, especially in the handling of fetal bones and bones of the child at birth. Here the parts must be cleaned, wiped, and weighed as rapidly as possible, or there is a marked variation, as these young bones have a greater percentage of inherent moisture. If the weighing process is slow, this variation will be noticed right on the balance pans.

By this method pieces of adult bone weighing from 1.1 grams up to over 3 grams were prepared. In fetal bones and those of the cat and rabbit, however, the weight of green bone was about 0.5 gram, as they are very bulky for their weight.

To procure and prepare four pieces of femur, tibia, and fibula and get the green weight of each requires about two hours of tedious and patient labor, but this is not the end.

The importance of the removal of all of the cancellous tissue and contained marrow will be shown in determinations A, B, C, and D. It will also show why cancellous bone proper should not be used, as the true organic constituency of osseous tissue cannot be correctly determined therefrom. This is due to the inability to get at and to remove the marrow from the cancelli.

Each of the four pieces of sample was treated as follows:

1. Green. The first piece of each bone was immediately calcined until the weight was constant and then the percentage of organic material determined, as will be shown later.

2. Oven-dried. The second piece of each bone after preliminary weighing was placed in an oven at 56°C. for twenty-four to forty-eight hours, allowed to cool, and then weighed. The loss in weight indicated the amount of moisture and the volatile organic substance present. It was then calcined until the weight was constant. By this method the percentage of moisture and volatile matter was found and also the amount of what might be termed real, or fixed organic material was obtained. In addition the green (original) weight permitted a determination of the organic material in green bone before the drying process was undertaken, giving a green-check determination.

3. Alcoholic-extracted and oven-dried. The third piece of each bone (after preliminary weighing) was placed in a 95 per cent

alcohol for twenty-four to forty-eight hours and then transferred to an oven at 56°C. for twenty-four to forty-eight hours, and then weighed when cooled. The weight lost indicated the moisture and volatile material and alcohol-soluble material. After calcining then the percentage of organic material in green bone, the percentage of moisture, volatile material and alcohol substance was next determined, and lastly the amount of remaining (fixed) organic substance.

4. Ether-extracted and oven-dried. In this determination a fresh piece from each bone (after preliminary weighing) was placed in ether from twenty-four to forty-eight hours, then oven-dried at 56°C. for twenty-four to forty-eight hours, and then weighed when cool. After calcination the percentage of organic material in green bone, the percentage of moisture, ether-soluble and volatile materials was next computed and then the amount of (fixed) organic substance remaining was computed.

The calcination was carried out by the use of two Bunsen burners. The porcelain crucible containing the green or extracted bone was placed on a triangle at an angle of about 45° and just high enough so that the light blue cone was close to the crucible. The crucible and contents were warmed gently and then the burner put in a position with some air cut off so that for the first half hour the heat would not be so intense, but sufficient to volatilize and drive off most of the carbon and volatile substances. These would ignite and burn at the mouth of the crucible. Following this, the air was turned on full to get the greatest heat and another Bunsen with all of the air turned on was held so that the blue cone was directed upon the piece of bone at the mouth of the crucible. Between these two flames the calcining was completed; the bone being turned from time to time. In this way the bone is rendered incandescent and all carbon is burned out. The time varies for different thicknesses from five minutes to half an hour. This is repeated until the weight is constant. The heat should be carefully applied at first.

In order to comprehend the following tables the method of determining these various percentages will be first given by an example:

Body 89—1920	Negro	Frozen	About 35 years of age	
	Ether—Oven-dried			
Bone No.				
Femur 90	31.6305	31.6305	4.0670 a	4.0670 a
	27.5635	27.9450	3.6845 b	2.4029 d
	4.0670 a	3.6845 b	0.3825 c	1.6641 f
	31.6305	3.6845 b	f/a = 40.90 per cent organic material in green bone.	
	29.2276	2.4029 d		
	2.4029 d	1.2816 e	c/a = 9.55 per cent moisture, ether-soluble substance, etc.	
			e/b = 34.78 per cent fixed organic material in the extracted bone.	
			e/a = 31.51 per cent of fixed organic material in reference to green bone.	

In all of the weighings a constant weight (50 grams) was used on the left pan and the right one contained the watch-glass, bone, and weights required to balance the 50 grams.

The various letters indicate the following:

- a = The weight of the green bone.
- b = The weight of the bone after (1) oven-drying or (2) alcohol-extracting and oven-drying or (3) ether-extracting and oven-drying.
- c = The weight of the moisture and volatile material alone or (1) + the alcohol-soluble or ether-soluble material.
- d = The weight of the calcined bone.
- e = The weight of the organic material after (1) oven-drying, or (2) after extracting and oven-drying.
- f = Organic material in green bone.
- f/a = The percentage of organic material and water, volatile and extracted material in green bone.
- c/a = The percentage of water, volatile and extractable material in green bone.
- e/b = The percentage of fixed organic material in the dried or extracted and dried bone.
- e/a = The percentage of fixed organic in the green bone.

These last two percentages exclude the water and volatile material, and while one percentage is in relation to the weight of the extracted bone, the other percentage is in relation to the weight of the green bone.

The results obtained will be tabulated with reference to age and method employed. *Fresh* indicates simple postmortem, not embalmed and not preserved in any way; *frozen* indicates storage

in the cadaver refrigerator and unembalmed; *dissection* refers to material taken from cadaver after the dissection was completed.

Green bone

Four and one-half months (fresh)

NUMBER	BONE	f/a	REMARKS
7	Fe	63.35	Fresh
8	Tib	66.05	

At birth (fresh)

1	Fe	49.61	Extremity of femur consisted mostly of cancellous bone and contained marrow and blood
2	Tib	50.44	
55	Fe	50.79	
56	Tib	53.07	
A		65.95	

20 to 60 years

84	Fem	41.15	Negro
85	Tib	41.95	47 years
84a	Fib	39.85	Frozen
112	Fem	40.61	Dissection
113	Tib	41.32	
113a	Fib	40.98	

61 to 90 years

11	Fe	40.88	71 years
15	Tib	42.46	Frozen
19	Fem	41.57	Very greasy
23	Feb	44.18	Fresh
24	Tib	44.68	Bones thin, 87 years
31	Fem	43.75	Frozen
35	Tib	45.25	
39	Fib	41.56	
43	Fe	42.28	Fresh. 76 years
44	Tib	41.40	
45	Fib	41.00	
63	Fe	48.62	80 years
64	Tib	45.77	Fresh. Bones thin and buckled in calcining
66	Fib	41.48	

Green Bone—Continued

61 to 90 years

NUMBER	BONE	f/a	REMARKS
76	Fe	39.81	Frozen. 87 years
77	Tib	41.68	
77a	Fib	40.71	
92	Fe	39.44	65 years
93	Tib	41.78	Negro. Frozen
100	Fe	43.39	Fresh. 89 years
101	Tib	39.86	
101a	Fib	40.80	

Oven-dried bone

Four and one-half months

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
9	Fe	61.23	27.41	46.59	33.82	Fresh
10	Tib	65.35	35.04	46.67	30.31	

Eight and one-half months and full term (at birth)

108	Fe	52.20	21.50	39.34	30.88	8½ months
109	Tib	53.01	22.04	39.70	30.98	Fresh
3	Fe	49.22	16.03	39.50	33.19	At birth
4	Tib	50.88	17.15	40.73	33.74	Fresh
57	Fe	52.20	19.34	40.72	33.00	At birth
58	Fib	54.95	25.52	39.55	29.46	Fresh
B		66.61	41.59	44.04	25.72	Extremity femur, mostly cancellous bone

20 to 60 years

86	Fe	40.57	8.36	35.15	32.20	Dissection
87	Tib	40.95	9.29	35.39	31.66	
87a	Fib	38.99	8.27	33.49	30.72	
114	Fe	40.28	9.22	34.21	31.06	
115	Tib	40.77	9.59	34.13	30.79	
115a	Fib	40.43	8.34	35.03	32.09	
122	Fe	42.31	7.71	37.01	33.80	
123	Tib	40.65	6.32	36.25	34.24	
123a	Fib	41.04	7.85	36.02	33.49	

Oven-dried bone—Continued

61 to 90 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
12	Fe	40.70	8.42	35.36	32.44	
16	Tib	40.48	lost	lost	lost	
16a	Fib	43.36	8.70	37.96	34.66	
25	Fe	43.63	9.86	43.51	39.65	
26	Tib	46.51	7.61	42.10	38.89	
32	Fe	42.28	3.48	40.11	38.66	
36	Tib	42.38	8.04	37.34	34.34	
40	Fib	40.00	6.85	35.58	33.14	
46	Fe	41.19	9.23	35.22	31.97	
47	Tib	42.41	11.55	34.61	30.62	
48	Fib	41.10	9.78	34.71	31.32	
67	Fe	51.50	15.16	42.52	36.10	Moisture very high, bones shrunk as they calcined
68	Tib	45.45	11.00	36.76	32.72	
69	Fib	41.85	27.76	19.49	14.82	
78	Fe	41.62	12.94	37.74	35.70	
79	Tib	39.76	7.81	34.67	31.96	
79a	Fib	39.20	5.79	35.29	33.39	
94	Fe	40.01	7.01	35.53	33.05	
95	Tib	39.22	6.80	34.77	32.41	
95a	Fib	40.09	7.82	35.15	32.48	
102	Fe	40.86	6.40	36.84	34.10	
103	Tib	41.42	6.59	37.29	34.83	
103a	Fib	41.44	7.94	36.39	33.38	

Alcohol-extracted and oven-dried bone

At birth

5	Fe	53.67	23.63	38.70	29.56	
6	Tib	53.81	30.08	39.46	27.58	
59	Fe	50.36	23.37	38.53	30.32	Extractives high. Can- cellous bone in one end of sample
60	Tib	50.60	19.88	38.34	30.74	
C		68.81	44.92	43.51	23.89	Extremity of tibia. Lots of extractives and mois- ture

Alcohol-extracted and oven-dried bone—Continued

20 to 60 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
88	Fe	39.66	6.28	35.22	33.00	Dissection
89	Tib	39.45	6.87	35.01	32.61	
89a	Fib	38.80	8.27	33.49	30.72	
116	Fe	40.62	8.99	35.09	32.19	
117	Tib	41.44	9.60	35.73	32.77	
117a	Fib	40.09	11.17	32.69	32.42	
124	Fe	42.85	8.35	37.75	34.61	
125	Tib	41.07	7.24	35.94	33.28	
125a	Fib	41.78	9.37	35.76	32.33	

61 to 90 years

13	Fe	40.01	7.34	35.21	32.66	Eccentric results
17	Tib	43.46	9.52	37.41	33.86	
21	Fe	41.41	5.53	37.98	35.58	
27	Fe	46.55	10.54	40.20	36.04	
28	Tib	47.36	10.55	41.40	36.21	
33	Fe	40.89	7.11	36.37	33.79	
37	Tib	45.01	9.49	39.25	35.53	
41	Fib	41.24	8.46	36.31	32.50	
49	Fe	41.27	9.88	34.83	31.38	
50	Tib	42.09	11.96	24.22	30.12	
51	Fib	41.71	10.73	34.69	30.89	
70	Fe	47.42	8.37	42.45	38.90	
71	Tib	44.77	15.41	34.71	23.78	
72	Fib	43.98	11.74	36.37	32.10	
80	Fe	41.02	6.90	36.60	34.13	
81	Tib	41.28	8.47	35.84	31.77	
96	Fe	40.20	7.47	35.48	32.85	
97	Tib	40.36	7.31	35.94	33.31	
97a	Fib	40.17	6.09	36.28	33.92	
104	Fe	41.04	8.08	35.86	32.19	
105	Fib	41.11	8.34	35.75	32.77	
105a	Tib	40.42	8.00	35.24	32.42	

Ether-extracted and oven-dried bone

Eight and one-half months and full term (at birth)

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
110	Fe	51.35	20.04	38.95	31.14	8½ months. Fresh
111	Tib	52.44	21.53	39.38	30.73	
61	Fe	53.56	23.85	31.35	29.71	
62	Hum.	54.48	23.35	40.43	30.91	Extremity of tibia mostly cancellous
D		66.57	41.54	41.68	24.96	

20 to 60 years

90	Fe	40.66	9.55	34.78	31.51	
91	Tib	41.63	10.40	34.88	31.25	
91a	Fib	39.46	8.01	34.19	31.45	
118	Fe	41.46	9.43	33.81	30.62	
119	Tib	39.56	9.01	33.94	30.87	
119a	Fib	39.49	8.32	33.36	31.17	
126	Fe	42.59	11.58	35.08	31.01	
127	Tib	41.32	8.18	36.66	33.23	

61 to 90 years

14a	Fe	41.84	8.14	36.69	33.70	Frozen 71 years
18a	Tib	41.98	7.67	36.85	33.86	
22	Fib	40.76	6.35	36.69	34.40	
29	Fe	42.11	9.03	35.23	32.47	87 years Extractives high
30	Tib	42.48	11.29	35.17	31.18	
34	Fe	46.62	8.77	34.29	27.86	
38	Tib	39.13	8.80	33.43	30.48	
42	Fib	44.70	15.59	34.49	29.09	
52	Fe	41.42	11.01	34.19	30.41	
53	Tib	41.25	11.64	33.52	29.61	
54	Fib	41.29	10.80	34.18	30.49	
73	Fe	42.85	15.23	32.58	27.62	
74	Tib	47.89	13.57	39.70	33.56	
75	Fib	43.00	15.64	32.54	27.41	

Ether-extracted and oven-dried bone—Continued

61 to 90 years

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
82	Fe	43.72	13.37	35.05	30.35	
83	Tib	43.16	13.26	34.47	39.89	
83a	Fib	41.45	12.43	33.15	29.04	
98	Fe	40.60	8.17	35.37	32.50	
99	Tib	42.31	10.24	35.73	31.71	
99a	Fib	40.28	9.04	34.36	31.25	
106	Fe	41.21	10.26	34.49	30.05	
107	Tib	41.11	10.40	34.82	30.71	
107a	Fib	40.61	9.45	34.01	31.26	

Cleaned bones of study sets

NUMBER	BONE	f/a	REMARKS
160	Fe	36.73	Quite clean, dry, apparently free from grease, the tibia had a little marrow and was greasy around the marrow cavity
161	Tib	36.66	
162	Fib	37.80	
163	Fe	36.15	Fairly clean
164	Tib	35.72	Clean
165	Fib	34.41	Very clean and dry, like ivory
166	Fe	39.70	Surface greasy to the touch
167	Tib	41.54	Very greasy to the touch. Only partly degreased in the boiling
168	Fib	42.08	

Green bones

Rabbit (two-third grown)

501	Fe	35.87	
505	Tib	41.74	
509	Fe	37.00	
513	Fe	37.66	
517	Tib	41.43	

Cat (adult)

530	Fe	39.43	
531	Tib	39.69	

Oven-dried bones
Rabbit (two-thirds grown)

NUMBER	BONE	f/a	c/a	e/b	e/a	REMARKS
502	Fe	35.11	7.85	29.59	27.76	
506	Tib	35.08	5.67	31.15	29.41	
510	Fib	38.90	10.16	31.98	28.74	
514	Fe	40.64	12.81	31.91	27.82	
518	Tib	42.07	12.99	33.37	29.04	

Cat (adult)

532	Fe	39.10	9.75	32.37	29.31	
533	Tib	39.01	8.75	33.13	30.24	

Alcohol-extracted and oven-dried bones

Rabbit (two-thirds grown)

503	Fe	38.78	11.79	30.64	26.98	
507	Tib	38.54	10.58	31.28	27.77	
511	Fe	34.97	9.82	27.88	25.14	
515	Fe	38.38	11.54	30.57	27.04	
519	Tib	40.35	11.33	32.61	28.86	

Cat (adult)

534	Fe	39.28	10.40	32.17	28.81	
535	Tib	39.49	9.08	33.70	30.75	
535x	Tib	43.88	13.03	35.65	31.09	Extremity containing considerable cancellous bone and marrow

Ether-extracted and oven-dried bones

Rabbit (two-thirds grown)

508	Tib	41.28	12.27	33.06	28.95	
512	Tib	38.25	9.38	31.88	28.87	
516	Fe	40.24	12.18	33.54	28.08	
520	Tib	41.80	12.56	33.55	29.38	

Cat (adult)

536	Fe	39.04	9.90	32.35	29.14	
537	Tib	38.32	9.60	32.14	29.22	
537x	Tib	42.09	11.29	34.01	30.79	Extremity of shaft with cancellous bone and marrow

DISCUSSION

Green bone

By making a green weight of all pieces of bone used for these determinations, a normal green-weight percentage of the organic material is obtained in all of the different methods of after-treatment. This not only gives a greater number of results (or a better average) for this determination, but also acts as a check for the other methods. In the opinion of the writer, this green determination is the one that should be used in referring to the organic and inorganic constituents of compact bone.

Fetal bone (four and one-half months)

Upon examining the results of the determinations upon green bone, some very interesting facts are brought out. In the fetus we find that the percentage of organic substance is very high (63.99 per cent), the bone practically consists of two-thirds organic material in the fetus at four and one-half months. This leaves one-third inorganic substance. This is natural, as the bone, before its ossification, is purely organic material. In making this examination great care had to be exercised in preparing the specimen and the weighing had to be rapidly made, as the drying upon the scale pan could be noted. Again, the piece of bone free from the cancellous tissue and marrow necessarily could not be large, as the bones at that age are small.

Bones at term (eight and one-half to ten months)

At eight and one-half months and birth we note that the average is 52.15 of organic material. This is an increase in the percentage of inorganic material with a variation from 49 per cent, or practically half organic and half inorganic substances. This variation, of course, is reasonable, as the diet of the mother has a great influence upon the hardness of the bones of the child. If the mother's diet be rich in bone-forming elements, no doubt the percentage of inorganic material would be raised beyond the above figures.

In this table is the determination of bone A. Here the percentage is 65.95. Why is it so high, practically that of the fetus of the fourth to the fifth month? The note in connection with the determination states that this specimen was the epiphyseal extremity of the diaphysis (in contradistinction to the central part usually used) and that it consisted mainly of cancellous bone with its contained marrow. The percentage is practically 15 per cent above the average—a variation too great to be overlooked.

It will be remembered that at the beginning of this paper it was stated that all cancellous bone was removed before the bone was weighed, because cancellous bone contains marrow which could not be removed independently of the bone, and its presence would increase the percentage of organic material if left in the specimen. Marrow is not an organic constituent of bone and must, therefore, be removed. If it is not got rid of, then an inflated per cent of organic content is the result. The determination in the fetal bone (A, table 1) is an excellent example and proof that if a specimen contains cancellous bone, it should be entirely discarded or the cancellous bone should be completely removed. Hence the care exercised in the preparation of all specimens before weighing. This same proportionate increase will be seen in the companion determination B, C, and D.

Green bone (20 years to 61 years)

In this group the average per cent is 40.75 of organic substance. These determinations are quite close. The lowest is 38.80 per cent and the highest is 42.85, while most of the determinations are between 40 per cent and 41 per cent. In reference to these variations it might be noted that the tibia giving 38.80 per cent was from an individual 47 years of age and all other green determinations ran along this same rate, all under 40 per cent, showing that the whole series was consistent and not an individual variation due to an accidental loss. In reference to the 42.85 per cent, it might be noted that the age was 60 (border line), and again all but one were above 42 per cent, again showing a consist-

ent figure and not an abnormal variation. Now this might be due to an unusually great amount of fat in the marrow and to unusually large haversian canals in the neighborhood of the marrow cavity, containing an unusually large amount of fatty marrow; or again, it might indicate a border-line case in which there is a slight increase of organic material in old age and in this particular individual this increase came on a little earlier than usual (an early senescence). In the next series the percentage of one case of 65 years varies between 39 and 40, and, therefore, resembles those of the younger group, indicating a comparatively youthful state.

Green bone (61 to 90 years)

In this group the ages are 65, 71, 76, 80, 87, and 89 years, giving a fairly varied series. Here we find the lowest to be 39.13 in one of 87 years. Determinations were made on the fresh subject and after the remains had been in the cold storage for quite a while; while in the fresh state, the green-bone percentages were consistently high, after freezing the percentages were consistently lower and more like 20 to 61 years of age. A variation in the moisture was also noted under these different conditions.

The highest was 51.50 and 48.03 per cent in two different individuals aged 80 and 87 years, respectively. These are isolated instances which might fairly be thrown out, but still they were retained and included in the series for the reason that this moisture and extractive content were relatively high in each case, seeming to indicate that these figures were not accidental, but in keeping with the other percentages in the experiments. In connection with most of the specimens of the individual aged 83 years, it might be mentioned that upon calcination the bones shrank, curled, or buckled and reacted like the fetal and animal bones where the organic content is relatively high or the bones are relatively thin.

Oven-dried bone

In order to try to obtain as complete a series of determination as possible, the moisture and volatile organic substances were first removed and then fixed the organic content determined. After the green weight was determined, then all of the desired specimens were subjected to the same heat (drying oven at 56°C.) to remove the moisture and ordinary volatile material (if any such were present). Then by weighing and subtracting the weight from the original green weight, the weight of the moisture and ultimately its percentage were readily determined. Then the amount or weight of the fixed organic material can be obtained. From the weight two results may be deduced: *a*) the percentage of organic substance to green bone and, *b*) the percentage of organic substance to the dried bone (green bone less its moisture). These three percentages will be taken in order.

Moisture and volatile organic material (c/a)

Four and one-half months. Here we find the percentage of moisture to average 32.43 per cent. This seems high, but we must remember that the bones are still two-thirds organic material, and organic material, in general, contains a large percentage of free water. We must also remember that the mesenchymal tissues at this stage are largely undifferentiated and so mainly embryonic in character and of a higher water content than at birth and later. We should, therefore, naturally expect a higher moisture percentage.

Eight and one-half months and full term. Here the average is 20.26 per cent with a variation from 16.03 to 25.52. By consulting the table it will be noted that the femur has a lower moisture content than the tibia, probably because the former is older from the developmental standpoint. It will also be noticed that in the eight and one-half months' fetus, the percentages are between those of the two full-term fetuses. This evidently again indicates a difference in the maternal diet. The specimen B represents the epiphyseal extremity of the diaphysis and it consisted mainly of cancellous bone and its contained marrow.

In the moisture content was 41.59 per cent due to the great quantity of blood that could not be removed. This shows why cancellous bone should not be used.

20 to 60 years. In this group while the average is 8.44 per cent, the highest is 9.59 per cent and the lowest 6.32 per cent. The highest amounts were usually noticed in the fresh (unfrozen or unembalmed bodies) and the lowest in those that had been frozen or embalmed. While there seems to be an extraction of moisture from the bones during storage in the refrigerator and an abstraction of moisture from the bones by the embalming fluid in many of the determinations, it is not constant, as will be pointed out later.

61 to 90 years. In this group there is a remarkable variation in moisture content. The average is 8.89 per cent, while the highest is 27.76 per cent and the lowest 3.48 per cent. The former occurred in the tibia of the 80-year-old individual, and this specimen curled and buckled when calcined. The lowest percentage (3.48) occurred in the 80-year-old individual after freezing, and yet other determinations of the same gave as high as 12.94 per cent. This body showed erratic results, so a number of determinations were made by various methods and all were included in the averages.

Although the average of moisture in the group 61 to 90 years is only 0.45 per cent higher than in the 20 to 60 years' group, still that little represents what the proportionate difference should be in comparing their green weights of 40.05 per cent and 42.24 per cent, respectively.

Organic material less water and volatile material in oven-dried bone

Having removed the water and volatile material, the weight of the calcined bone may now be compared with the original green weight and also with the oven-dried weight.

Relations of the organic (less moisture) to oven-dried bone (c, b)

Four and one-half months. If now we determine the percentage of organic substance after removing the water in the oven-dried bone, the average is 46.63 per cent.

Eight and one-half months to full term. In this group the average is 39.92 per cent with a variation from 39.34 per cent to 40.73 per cent—a fairly uniform series.

20 to 60 years. In this group the average is 34.52 per cent with a variation from 33.49 to 37.01 per cent. The former occurred in an individual of 35 years and the latter is one of 60 years.

61 to 90 years. In this group the average is 36.22 per cent with a variation from 19.49 per cent to 43.50 per cent. The cause of the extreme variation could not be determined and, strange to say, the average of these two extremes is nearly the general average.

Relation of organic material (less moisture) to the green weight (e/a)

Four and one-half months. In this group the average is 32.06 per cent. By adding this to the percentage of moisture, the result is 63.49 per cent of combined moisture and organic substance proper. The amount given is 63.29 per cent as per the table. All these percentages were obtained by dividing one number into the other and not by mere subtraction, hence the sum of e/a and c/a are not always equal to f/a as they would be by mere subtraction.

At eight and one-half months and full term. The average here is 31.88 per cent with a variation from 29.46 per cent to 33.74 per cent.

20 to 60 years. In this group the average is 32.23 per cent with a variation from 30.72 per cent to 34.24 per cent. The highest were those in the individual of 60 years, the lowest in those of 35 years.

Alcohol extracted and oven-dried bone

This series consists of the results of treating green bone with alcohol for twenty-four to forty-eight hours and then drying for twenty-four to forty-eight hours in an oven at 56°C. and weighing to determine the amount of moisture and alcohol-extractable substances and later the 'fixed' organic substance (in relation to the green weight and the extracted weight).

Moisture and alcohol soluble extract (c/a)

At full term. The amount of moisture and alcohol-soluble material averages 24.24 per cent in the child at birth. The lowest is 19.88 per cent and the highest 30.88 per cent. This average represents 3.98 per cent more than the mere moisture and volatile material in oven-dried bones. This does not indicate a high percentage of soluble material, indicating some little fat or oil in the compact bone proper.

20 to 60 years. In this group the average is 8.46 per cent of moisture and alcohol-soluble material. The extremes are 6.28 per cent and 11.17 per cent. The average highest is in those bones of the individual of 35 years of age, while in the subject of 60 years of age they are intermediate. The average is almost identical with the average per cent of plain moisture and volatile substance (8.44), arguing that in individual cases there may be a difference, but in the main (average) that the alcohol-soluble material is very small in quantity.

61 to 90 years. In this group the average is 9.01 per cent with variations from 5.53 per cent to 14.51 per cent. This is higher than the preceding by a half per cent and is only 0.12 per cent above the plain moisture of the oven-dried group. Evidently in the adult the compact bone contains exceedingly little alcohol-soluble substance.

Fixed organic material in alcohol-extracted and oven-dried bone
Relation of the organic substance to the extracted and
dried bone (e/b)

At full term. In this group the average is 38.76 per cent. This is only 1.16 per cent less than the same determination in plain oven-dried bone.

The average here is 29.55 per cent. This is the real organic substance in compact bone of this age. This is 1.51 per cent lower than that of the plain oven-dried bone, indicating some alcohol soluble material in the bone at birth.

20 to 60 years. In this group the average is 32.66 per cent with variations from 30.70 to 34.61 per cent. This is 0.69 per cent

lower than that of oven-dried bone, meaning that there is very little alcohol-soluble substance in compact bone.

61 to 90 years. In this group the average is 32.67 per cent with variations from 23.78 to 38.90 per cent. Compared with the corresponding determination in oven-dried bone, it is 0.59 lower than that.

Ether-extracted and air-dried bone moisture and ether-soluble substance (c/a)

At term. The average of moisture and ether-soluble substance in compact bone at birth averages 22.19 per cent with variation of 20.04 per cent to 23.85 per cent. This is 2 per cent lower than alcohol-extracted bone and about 2 per cent higher than mere oven-dried term bones.

20 to 60 years. In this group the average is 9.27 per cent with variations from 8.18 to 11.58 per cent. This average is 1.19 per cent higher than alcohol-extracted bone and 1.21 higher than oven-dried bone.

61 to 90 years. In this group the average is 10.87 per cent with variations from 8.17 to 15.60 per cent. This average represents 1.86 per cent and more than that of alcohol extracted bone and 1.98 per cent more than oven-dried bone.

Relation of fixed organic material in ether-extracted bone to ether-extracted and oven-dried bone (e/b)

At term. This average is 37.53 per cent as compared with 38.76 in the alcohol-extracted bone and 39.92 per cent in oven-dried bones.

20 to 60 years. The average in this group is 34.46 per cent as compared with 35.07 per cent in alcohol-extracted and 36.52 per cent in oven-dried bones.

61 to 90 years. Here the average is 34.83 per cent as compared with 37 per cent in alcohol-extracted, and 46.22 per cent in plain oven-dried bones.

Relation of fixed organic material in ether-extracted bone to the green weight (e/a)

At term. The average is 30.60 per cent as compared with the average of 29.55 per cent of alcohol-extracted bone and 31.88 per cent in oven-dried bones.

20 to 60 years. The average is 31.34 per cent as compared to 32.66 per cent in alcohol-extracted bones and 32.23 per cent in oven-dried bones.

61 to 90 years. The average is 30.82 per cent in this group. This is to be compared with 32.67 per cent in alcohol-extracted bones and 33.26 per cent in oven-dried bones.

Cleaned bones

In order to make the study as complete and varied as possible, it was decided to take some of the bones of the study collection sets and determine the organic content of so-called cleaned bones. For this purpose three different sets were chosen, a piece of femur, tibia, and fibula in each set to conform to the preceding tests. One set was as clean and dry as possible (A), a set that showed grease and was greasy to the touch, yet cleaned as the others had been (C), and, thirdly, an intermediate set that looked fairly clean and yet gave indication of some grease (B).

B

NUMBER	BONE	f/a	REMARKS
160	Fe	36.73	These seemed quite clean and were apparently free from grease. The tibia contained a little dried marrow and was greasy around the marrow cavity
161	Tib	36.66	
162	Fib	37.80	

A

163	Fe	36.15	The femur was fairly clean
164	Tib	35.72	The tibia was quite clean looking
165	Fib	34.41	The fibula was very clean and dry and looked like ivory

C

166	Fe	39.70	The surface of the femur was greasy to the touch. The tibia and fibula were very greasy to the touch and only partly degreased in the cleaning
167	Tib	41.54	
168	Fib	42.08	

In the test upon the fresh uncleaned bones the corresponding results are as follows:

(e/b) in oven-dried bones:		<i>per cent</i>
20 to 60 years		34.52
61 to 90 years		36.22
(e/b) in alcohol extracted and oven-dried bones:		
20 to 60 years		35.07
61 to 90 years		37.00
(e/b) in ether-extracted and oven-dried bones:		
20 to 60 years		34.46
61 to 90 years		34.83

In examining these results, interesting facts are noted. In group A, although the bones seemed clean, dry, and free from grease, the results varied. The tibia was the best looking and showed that all of the soluble and volatile substance had been removed and only the fixed organic material had been left. The percentage of fixed organic material in this bone was 34.41 per cent. This is practically identical with the percentage of fixed organic material in the ether-extracted bone and seems to indicate the complete extraction of all but the fixed organic substance. As we look through the other percentages we see the effect of the presence of unnoticeable and distinctly noticeable quantities of grease that remain through the incomplete cleaning of bones. Naturally, any such retained grease will have weight and will necessarily be included in percentage of fixed organic substance, though it should not be so included.

Another interesting fact is that in cleaning bones by boiling or macerating in water alone, all bones of the same body do not degrease or clean equally. Again some bones on repeated treatment seem to retain the grease to a considerable extent.

From this series it will readily be granted that the ordinarily cleaned bones are not satisfactory as a standard for the determination of organic substance in compact bone for bones that seem dry, clean, and free from grease may contain 1 or 2 per cent, and so change the final determination.

Other mammals

As some rabbits and cats were available at the time these determinations were made, it was decided to carry out the same series of tests upon the corresponding bones of these animals. The bones were prepared and treated in the same way and the tests were identical in all respects. The bones of these animals, however, are more difficult to handle. They are thin and bulky so that the amount of bone by weight was not great, varying from 0.1 gram to 0.5 gram. This did not allow a very great leeway for variations. Another prominent feature was that the bones at the first heating curled and split; and if the heat was not cautiously applied, small pieces might readily fly off and be lost. Also at the first heating the small areas of the bone that touched the crucible seemed to stick, but this did not seem to make any appreciable variation in the result.

The bones clean readily, as the periosteum strips with ease and the marrow comes out in toto as a plug would. There is no cancellous bone around the narrow cavity to be whittled away as in the human bone, so that the preparation of these bones is not so tedious a process as in the case of the human bones.

The weighing, however, must be rapidly carried out, as in the case of fetal bones, for these bones seem to contain a little higher percentage of moisture (as fetal bones) than the adult human bone. Perhaps this is only apparent and their thinness gives a greater proportionate surface for evaporation and so influences the result. There is no difficulty in getting the real green weight, however.

Rabbit. The rabbits used were all about two-thirds grown and represent the human at about the age of puberty. They were all in good health and were well developed. They had been utilized for other experimental work, but this had no influence upon the tests in hand.

Green bone. In this group the average of all of the bones tested was 38.90 per cent of organic substance, somewhat lower than we would expect to find it at the corresponding age of the human being.

The other determinations will be considered as follows:

Moisture and volatile and extractable substances

In oven-dried bone the average was 9.89 per cent.

In alcohol-extracted and oven-dried bone the average was 11 per cent.

In ether-extracted and oven-dried bone the average was 11.60 per cent.

Relations of the fixed organic substances to the extracted and dried bone (e/b)

In oven-dried bone the average was 31.60 per cent.

In alcohol-extracted and oven-dried bone the average was 30.58 per cent.

In ether-extracted and oven-dried bone the average was 33.01 per cent.

Relation of the fixed organic substance to the green bone (e/b)

In oven-dried bone the average was 28.56 per cent.

In alcohol-extracted and oven-dried bone the average was 27.16 per cent.

In ether-extracted and oven-dried bone the average was 28.82 per cent.

Cat. In the course of the laboratory work several cats were used for tissue purposes and so at the time the corresponding bones were prepared, as in the human, for these determinations. They were of adult age, well developed and apparently healthy.

Green bone. The average of all the bones tested was 38.32 per cent—somewhat low—even than the corresponding average in the human being.

Moisture, volatile, and extractable substances (c/a)

In oven-dried bone the average was 9.25 per cent.

In alcohol-extracted and oven-dried bone the average was 11.01 per cent.

In ether-extracted and oven-dried bone the average was 11.60 per cent.

*Relation of fixed organic substance to the extracted and dried bone
(e/b)*

In oven-dried bone the average was 32.75 per cent.

In alcohol-extracted and oven-dried bone the average was 33.84 per cent.

In ether-extracted and oven-dried bone the average was 32.83 per cent.

*Relation of fixed organic substance in extracted bone to green
bone (e/a)*

In oven-dried bone the average was 29.77 per cent.

In alcohol-extracted and oven-dried bone the average was 30.22 per cent.

In ether-extracted and oven-dried bone the average was 29.72 per cent.

Having compared the results obtained by the different experimental methods, it will now be of interest to consider the determinations from another standpoint, according to the following tables: Here only the adult human results and cat and rabbit will be considered.

	<i>per cent</i>
Average in green bone at 20 to 60 years	40.75
Average in green bone at 61 to 90 years	42.32
Average in green bone in rabbit	38.90
Average in green bone in cat	39.85

Moisture and extractable substance (c/a)

Average in oven-dried bone 20 to 60 years	8.44
Average in oven-dried bone 61 to 90 years	10.18
Average in oven-dried bone, rabbit	9.89
Average in oven-dried bone, cat	9.25
Average in alcohol-extracted bone, 20 to 60 years	8.46
Average in alcohol-extracted bone, 61 to 90 years	9.01
Average in alcohol-extracted bone, rabbit	11.01
Average in alcohol-extracted bone, cat	10.84
Average in ether-extracted bone, 20 to 60 years	9.27
Average in ether-extracted bone, 61 to 90 years	10.87
Average in ether-extracted bone, rabbit	11.60
Average in ether-extracted bone, cat	10.26

Relation of fixed organic substance to dried and extracted bone (e/b)

Average in oven-dried bone, 20 to 60 years.....	35.17
Average in oven-dried bone, 61 to 90 years.....	36.22
Average in oven-dried bone, rabbit.....	31.60
Average in oven-dried bone, cat.....	32.75
Average in alcohol-extracted bone, 20 to 60 years.....	35.07
Average in alcohol-extracted bone, 61 to 90 years.....	37.00
Average in alcohol-extracted bone, rabbit.....	30.58
Average in alcohol-extracted bone, cat.....	33.84
Average in ether-extracted bone, 20 to 60 years.....	34.16
Average in ether-extracted bone, 61 to 90 years.....	34.83
Average in ether-extracted bone, rabbit.....	33.01
Average in ether-extracted bone, cat.....	32.83

Relation of fixed organic substance to the green bone (e/a)

Average in oven-dried bone, 20 to 60 years.....	32.23
Average in oven-dried bone, 61 to 90 years.....	33.15
Average in oven-dried bone, rabbit.....	28.56
Average in oven-dried bone, cat.....	29.77
Average in alcohol-extracted bone, 20 to 60 years.....	32.66
Average in alcohol-extracted bone, 61 to 90 years.....	32.67
Average in alcohol-extracted bone, rabbit.....	27.16
Average in alcohol-extracted bone, cat.....	30.22
Average in ether-extracted bone, 20 to 60 years.....	31.34
Average in ether-extracted bone, 61 to 90 years.....	30.82
Average in ether-extracted bone, rabbit.....	28.82
Average in ether-extracted bone, cat.....	29.72

Green weight (f/a)

In this table the average amount of organic substance and moisture in green bone (between 20 and 60 years) is 40.75 per cent. This is 6 per cent to 7 per cent higher than that given in text-books. Of course we must admit that this includes moisture and volatile substance and perhaps a very small amount of fat or oil that could not be removed by mere physical means. The specimens were prepared as carefully as possible, and the writer feels that the bone so prepared is standard for all green-weight determinations. The question naturally arises, "Should we include moisture and volatile substances in the per cent of organic material?" Just where to draw the line seems impossible to determine. The small quantities of fat and marrow in

perimedullary haversian canals cannot be removed in this method and they are counted in as part of the organic constituent, and yet they should not be and we are helpless in any effort to remove them.

Again in comparing the results at the prime and after, we find that in the latter instance the average is 42.32 per cent—somewhat higher than in the preceding. This argues for a slightly smaller inorganic content and would argue in favor of the reduction of inorganic as being a factor in the readiness with which the bones of the old fracture made only slight strains. Although the reduction is not so great as to give positive assurance of this, still we must take it into consideration as a possibility. More determinations along this line will be made as the material presents itself.

In comparing the results in the human and in the rabbit (two-thirds grown) we note that in the latter the average percentage of organic material is 38.90 per cent. This indicates immaturity in development and would compare favorably with like stages in the human being, no doubt. Unfortunately, no material was obtainable in individuals from birth to 20 years. From the closeness in the averages, no doubt the per cents in the adult rabbit came very close to that of the human being—showing a close relation in mammals apparently.

In the adult cat the percentage was 39.85—less than 1 per cent below that of the human. If as many determinations had been made in the cat as in connection with human bone, the writer believes that the difference would have been even less, indicating a close relationship in mammals with reference to the composition of compact bones.

Moisture and volatile and extractable substance (c/a)

In this table the average moisture in compact bone in those 20 to 60 years of age is given as 8.44 per cent.

The temperature (52°C.) is not sufficiently high to cause any decomposition, nor was it employed long enough to cause any untoward effects upon the above. Whether anything else

besides moisture is driven off or not was not determined, as that was beyond the province of this paper. This amount of moisture strikes one as quite high, yet when we consider that all of our organs and tissues are principally water (mainly combined) we should not be surprised at these results. It is variable in different individuals, as the detailed results in the earlier tables show. If this be not considered normal organic content, but be subtracted from the green weight, then the average, as will be seen later, gives what is normally called 'fixed organic substance.'

Between the ages of 61 and 90 the percentage of moisture, etc., is 10.18 per cent, and as we proceed in this analysis we will note that the increase in these ages is constant. The difference between these age groups is only 1.75, yet that is a definite increase.

In the rabbit the average moisture, etc., is 9.89 per cent, and this does not seem excessive for the proportionate growth, as the percentage in the young is greater than in the adults and then in old age seems to increase again.

In the cat the amount of moisture is 9.25—nearly 0.75 per cent greater than in the adult man. This is possibly a normal condition for that animal.

In the alcohol-extracted bone the percentage is almost identical with that of oven-dried bone, being only 8.46 per cent. It seems that compact bone prepared in the way for these determinations contains very little alcohol-soluble substance. In those of 61 to 90 years the percentage is only 9.01—below the weight of ordinary moisture. This variation cannot be accounted for.

In the rabbit the average is 11.01 per cent and in the cat 10.84 per cent, showing over 1 per cent increase in both instances. Again, the relatively higher percentage in the rabbit indicates a more youthful state.

The ether-extractable substance is greater. In the human bone 20 to 60 years the average is 9.27—a material increase over moisture and alcoholic extract. This increase is constant in all. In the rabbit the average is 11.60 per cent, in the cat 10.26 per cent. In all of these forms of animals there is a higher percentage

of ether-extractable substance, and this probably is the fat in the perimedullary haversian canals. This amount of ether-extractable substance is material in relation with total amount of moisture, but in reference to the total amount of organic substance it is not so marked. We must, however, realize that there is some ether-soluble organic material even in the compact bone and something which is difficult to classify, so the question arises, "Should it be included in the organic constituent or not?"

Relation of fixed organic substance to the dried and extractable bone (e/b)

As has been seen, the percentage of organic substance in green bone includes the moisture and soluble substances, and this naturally increased that percentage. Should they be included as organic constituents or excluded? If we exclude them, then we have remaining the real fixed organic substance and then we can deduce two different answers: 1) The relation of this fixed organic substance to the weight of the extracted bone (green bone less moisture and extracted substance e/b): The relation to the original green weight (without the removal of any moisture, etc., e/a).

Of course, the percentage in reference to the extracted bone weight (e/b) will be the greater and we find that the percentage in those 20 to 60 years is 35.17 per cent. This is a per cent or so above that given in text-books, but we are not told the method of determination, and the writer for one, does not believe that this was the method used. In those of 61 to 90 years the average is 36.72 per cent—again higher than in the earlier years. In the rabbit it is 31.60 and in the cat it is 32.75 per cent—considerably lower than in the human bone.

In the alcohol-extracted bone in those 21 to 60 years, the average is 35.07 per cent, while in those 61 to 90 years, the average is 37 per cent. Again, this determination shows less alcoholic extract than mere moisture as in the last table. This seems to indicate that alcohol may fix and render insoluble or non-volatile

some substances in the bone that are volatile with mere heat or soluble in ether. So the figures would argue at any rate. In the rabbit the average is 30.58 per cent and in the cat 33.84 per cent. In the ether-extracted bone, 20 to 60 years, the average is 34.16 per cent of organic substance as compared with the extracted weight of the bone. At 61 to 90 years the average is 34.84 per cent in both instances—a drop over the corresponding moisture and alcohol-extracted percentages. This is in keeping with the relatively higher amount of ether-extractable material, and should be so. In the rabbit the average is 33.01 per cent and in the cat 32.83 per cent. These are properly proportionate results.

Relation of the fixed organic substance to the green weight (e/a)

In these results the percentages should be lower than in the preceding, as we start with a higher original weight, including therein all moisture and extractable substances. In those 20 to 60 years of age the average is 32.23 per cent and in those 61 to 90 years the average is 33.15 per cent.

In the rabbit the average is 28.56 per cent and in the cat 29.77 per cent.

These percentages could all have been got by merely subtracting the percentage of moisture from the original average in green bone, but this was not done, but each was determined from the resulting weights, so in some instances the sum of the moisture percentage and this last per cent (e/a) does not always give the exact per cent of organic substance in green bone, but the per cent according to the figures obtained. We note in all these last determinations a constant and normal difference from the preceding determination.

In the alcohol-extracted bone, 20 to 60 years, the average is 32.66 per cent and in those of 61 to 90 years the average is 32.67 per cent—almost identical. In the rabbit the average is 27.16 per cent and in the cat 30.22 per cent.

In ether-extracted bone, 20 to 60 years, the average is 31.34 per cent and from 61 to 90 the average is 30.82 per cent. In

the rabbit the average is 28.82 per cent and in the cat 29.22 per cent. These are all consistent variations and will be noted by consulting the corresponding table in the other methods of determination.

CONCLUSIONS

Five methods for the determination of organic content of bone have been here given. Of these the ordinary method of cleaning (maceration) should be avoided, as results cannot be consistent owing to the irregularity with which bones of the same individual degrease.

All of the other four methods are constant and reliable, and every one may be considered as a standard. Various points for discussion arise. Should the moisture be included in the organic content? Should any alcohol- or ether-soluble substance be included in the organic content?

If we examine the tables, we find that the only percentages close to those given in text-books are those obtained in ether-extracted bone and in relation to the weight of the bone after extraction by the ether (e/b).

This may have been the method employed by the early chemists. It was employed here to try to determine the nearest to the text-book percentages. This determination (e/b) may seem unfair because it first removes the soluble and volatile substances and takes the weight of this dried bone as a standard to determine the fixed organic substance. It does not seem fair, but it discriminates against volatile and extractable substance. It can be argued that this moisture and this soluble material are real organic contents and that they should be so included just as in making an assay of ore, the whole sample is crushed and mixed for test and not just certain parts selected. In the latter instance any desired results could be obtained and these would not be the true or fair results. If the moisture and soluble material be considered organic content, then our text-books are incorrect to the extent of 6 per cent to 7 per cent. If, on the other hand, they are excluded and the percentage still be

determined in the green weight, then the text-book percentage is 2 to 3 per cent too high. Even in oven-dried bone the percentage in reference to the dried weight is still too high—37.15 (20 to 60 years) and 36.22 (61 to 90 years).

The writer would be inclined to consider the green weight the proper one for starting. After the bone has been freed of all periosteum and cancellous tissue and surface oil, its weight should be the one used, and the ultimate results would come under the determinations f/a , in which the average is 40.75 per cent (20 to 60 years) and 42.32 per cent (61 to 90 years).

The writer would like to have the opinion of others along this line so as to establish a standard for determination of the organic content of compact bone. Further studies in this connection are under way.

Resumen por el autor, Eben J. Carey.

Estudios sobre la estructura y función del intestino delgado.

- I. La arquitectura helicoidal del intestino delgado.
- II. La acción espiral del intestino delgado.

La capa muscular interna del intestino delgado es una lámina continua arrollada en espiral apretada. Una vuelta completa de espiral tiene lugar en cada 0.5 a 1 mm. de longitud, o menos. La capa muscular externa forma una espiral alargada, que termina una vuelta completa en una extensión de unos 200 a 500 mm. o más. La submucosa está compuesta de fibrillas de tejido conjuntivo que forman una espiral interna apretada y otra externa más laxa. La interna efectúa una vuelta completa en 0.5 a 1 mm. o menos, la externa en cada 4 a 10 mm. La capa muscular interna, por consiguiente, está arrollada en espiral apretada, la externa en espiral laxa.

La diferencia en la velocidad de la progresión translatoria de las dos ondas de contracción depende de esta disposición en espiral. La onda que marcha a lo largo del grupo interno de fibras toma un curso rotatorio, mientras que la que camina a lo largo de las fibras externas sigue una dirección más translatoria hasta alcanzar su punto de destino. Por consiguiente, la contracción de la capa muscular interna, más fuerte, seguirá inevitablemente a la de la capa externa. La disposición de las capas musculares intestinales explica claramente el fenómeno de la contracción cefálica y la dilatación caudal durante la diastalsis, sin necesitar invocar la ayuda de vías nerviosas hipotéticas. La peristalsis, por consiguiente, es un fenómeno de doble contracción producido por la velocidad diferencial del avance translatorio de las dos ondas de contracción en las capas musculares externa e interna, respectivamente. Estas conclusiones se basan en experimentos en los cuales el autor separó del intestino vivo las capas externa e interna.

STUDIES ON THE STRUCTURE AND FUNCTION OF THE SMALL INTESTINE

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TWENTY-TWO FIGURES

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1. THE HELICOIDAL ARCHITECTONICS OF THE SMALL INTESTINE

Introduction

The current literature and text-books of anatomy and the related medical sciences describe the two muscular coats of the small intestine as "inner circular and outer longitudinal." The inner muscular coat is conceived, therefore, as forming a tube composed of discrete muscular rings with a certain degree of connection. Such a conception is a faulty anatomical heirloom and, due to its apparent truth by a mere cursory microscopical observation, is accepted by the present generation as an assimilated fact. This erroneous idea arose with the inception of the microscope and has since been accepted unchallenged. Mall ('96) demonstrated the spiral nature of the submucosa twenty-

five years ago, but this important observation has remained as an obscure isolated report. The fact that the musculature possesses also a spiral architectonic did not occur to him.

The truth in regard to the form of a muscular structure is impossible when the evidence rests on microscopic observations of single sections. Painstaking reconstructions of serial sections may be an aid, but only when it is impossible to make gross dissections. The pitfall presented by the microscope reminds one of the following wise admonition given by John Goodsir ('46) to his freshman class in anatomy, "As soon would the astronomer place the telescope in the hands of his pupil and request him to interpret the sinuous lines by which the orbits of the planets are projected on the apparent surface of the hollow sphere, before he has acquired steady ideas of astronomical forms and motions by preparatory study, as would the judicious teacher of anatomy suggest the examination of objects by the microscope before strict anatomical ideas of form and relation had been acquired by the study of the gross specimens of the bones, viscera, muscles and blood vessels."

Studies on the interaction of the intestinal components (Carey, '19, '20 a; '20 b; '20 c) during the genesis of these two muscle coats reveal the following facts: The primitive gut presents two zones of differential rates of growth. There is an inner epithelial tube, growing in the manner of a left-handed spiral, of accelerated growth. Secondly, there is the outer mesenchyme, of retarded growth, from which the two muscle coats are derived. During the period of rapid transverse growth of the inner tube, the inner muscle coat is seen to become progressively drawn out by traction. This coat, then, by compression, restricts the diametrical growth of the entodermal tube. The planes of mitosis shift from a parallel to a transverse position as regards the long axis of the gut and the subsequent growth of the inner tube is relatively more rapid in length than in width. During this rapid longitudinal growth, the outer mesenchymal cells are drawn out by traction, revealing the first step in histogenesis, namely, an elongation of the myoblastic cell.

Since the tension in the mesenchyme corresponds to the spiral lines of the dominant growth-force, of the entodermal epithelial tube, the mature structure, as the muscular resultant of this interaction of growth and resistance, under no circumstances could possess a circula form. This assertion is based purely on embryological evidence. The confirmation of this evidence led to the following observations of gross dissections.

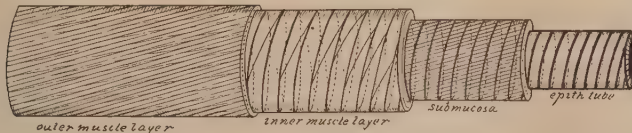


Fig. 1 Schema of the left-handed helicoidal structure of the small intestine based on dissections. The mitosis of the epithelial tube follows the path of a left-handed helix. Mucous folds are directed the same as the mitotic paths. This is seen in the valvulae conniventes which primarily form left-handed interdigitating spirals. This is readily demonstrated by everting the duodenum or jejunum. The submucous connective tissue forms inner close spirals and outer elongated spirals. This same arrangement holds for the muscular coats. The fasciculi of the muscular coats and the fibers of the submucous coat are connected by anastomosing elements, respectively. Muscular action based on this arrangement, therefore, is in the manner of a screw corresponding to the direction of the muscular fibers.

Direct observations on the helicoidal structure of the muscle coats of the small intestine

The small intestine of the pig is everted over a test-tube. By making a longitudinal incision through the mucosa and the submucosa these coats are then easily dissected from the inner muscular layer. Once the inner muscular layer is thoroughly cleaned of submucous and areolar tissue, a single smooth muscular fasciculus may be easily isolated and traced around the test-tube. By this means one may demonstrate incontestably that the fasciculi of the inner smooth muscle coat are wound closely in a compact spiral (fig. 1).

The main point to keep in mind in regard to this arrangement in the adult is that it reflects its embryonic origin. The muscle is derived from an embryonic syncytium which had been whorled

into a vortex. Consequently, there is bound to be intercommunications found between the spirally arranged fasciculi in the mature state.

In many instances one may trace a well-developed smooth muscle fasciculus in the duodenum or lower ileum near the cecum as a definite entity through ten or fifteen turns. This demonstrates that the inner intestinal muscular coat is not composed of discrete contiguous rings, but is a continuous syncytial muscular layer spirally wound anticlockwise.

By inserting a test-tube in the lumen of the intestine and dissecting off the serosa it may be demonstrated that the fasciculi of the outer coat do not run a straight longitudinal course, but run slightly in an oblique direction. In many instances a definite fasciculus or group of fasciculi may be traced from one side of the intestine to the other. There is a tendency to obliterate the spiral nature of the outer coat due to the torsional reaction of the intestine. This is manifested by the formation of loops which tend to counteract the strain to which the intestine is subjected in the spiral development of the entodermal epithelial tube. At the same time, this reaction tends also to counteract the spiral course of the outer layer.

The inner layer makes a complete turn in every 0.5 to 1 mm. or less; the outer layer makes a turn in every 200 to 500 mm. The definite obliquity of this outer layer is best detected after cleaning thoroughly along the line of the mesenteric attachment. It is then clearly demonstrable that the outer fasciculi do not run in a longitudinal course parallel to this landmark, but at an acute angle from right to left (fig. 1).

The spiral nature of the connective tissue of the submucosa may be demonstrated by inserting a test-tube in the lumen of the small intestine and dissecting off the serosa and musculosa. By allowing the submucosa and mucosa to incubate at 37°C, autolysis will occur, leaving the fibers intact. As desiccation proceeds, the fibers adhere to the test-tube, forming two definite spiral arrangements. There is an inner close spiral and an outer elongated spiral both wound anticlockwise or as a left-handed helix.

Mall ('96) detected the spiral nature of the submucosa, but described it as composed of fibrillae running in opposite directions, forming a spiral lattice-work. The observation of the spiral nature is correct, but the fibrils are arranged in two sets running in the same direction. The inner spiral is wound closely, the outer is more elongated. The latter makes one complete turn every 4 to 10 mm. the former in every 0.5 to 1 mm. or less (fig. 1).

Conclusions

1. The inner muscle coat of the small intestine is not composed of circular or annular rings contiguously placed, but is a continuous muscular sheet wound into a close helix. One complete turn is made in every 0.5 to 1 mm. or less.

2. The outer muscle coat of the small intestine is not composed of longitudinal fibers parallel to the long axis, but of elongated fibers, at an acute angle to the long axis of the intestine, which tend to pursue a spiral course. This spiral character is obliterated in many places, due to the torsional reaction of the intestine. Those which are spirally disposed make a complete turn in every 200 to 500 mm. or more.

3. The submucosa is composed of connective-tissue fibrils forming an inner close and an outer elongated spiral. The inner makes one complete turn in every 0.5 to 1 mm. or less, the outer in every 4 to 10 mm.

4. Therefore, the structure surrounding the epithelial tube of the intestine in the mature state possesses a spiral nature arranged as a left-handed helix with an inner close and an outer elongated set of spirals in both the submucosa and musculosa.

5. The valvulae conniventes of the duodenal epithelium are arranged as interdigitating spirals.

6. In the adult, consequently, confirmatory evidence is presented substantiating the thesis that the left-handed helicoidal growth of the epithelial tube creates a corresponding mesenchymal vortex. From the latter the spiral structure of the submucosa and musculosa is developed. This competition between the accelerated growth of the entodermal tube and the

retarded growth of the surrounding mesenchyme leading to a formative interaction of differential growth has been overlooked as a factor in histogenesis.

2. OBSERVATIONS ON THE SCREW-LIKE ACTION OF THE SMALL INTESTINE

Introduction

Since the direction of muscular contraction is dependent on the form and arrangement of the muscular fasciculi, it will be profitable to study intestinal action in the light of the spiral architectonics of the musculature. Form and function are inseparable; consequently, a contribution to anatomical structure is an aid to understanding its related function.

The study of the reciprocal elongating actions of the two muscle coats of the intestine presents one of the most confusing chapters in the entire literature of anatomy and physiology. Prior to the graphic records obtained by Bayliss and Starling ('99 a) it was thought that the outer and inner muscle layers contracted alternately by Ludwig ('61), Englemann ('71), Nothnagel ('82), Luderitz ('89), Mall ('96 a). Bayliss and Starling proved that these muscle layers contract simultaneously. But they interpreted their classical records by stating that contraction takes place in the two muscular layers in the same place at the same time. Although contractions begin in the two layers at the same position at the same time, the typical peristaltic resultant is at different locations in successive units of time. This will be proved to be inevitable because of the arrangement of the two sets of muscular fasciculi.

There are three main types of movement seen in the small intestine. First, there is a rapid vermicular wave. This is primarily seen after death or in animal experimentation. It progresses through the course of the small intestine in about a minute. It is considered by Mall ('96 b) as being more pathological than physiological. Second, there is the slow-moving peristaltic wave called by Cannon ('12 a) diastalsis. This normal wave propels the food through the small intestine in three to five hours. Third,

there is seen rhythmic segmentation which may or may not be combined with peristalsis. By combination of the two movements there is a slow advance of the food combined with definite constrictions of the intestinal contents. There is a rhythmic repetition of the segmentation. Normally, the wave progresses from above downward.

The slow peristaltic wave presents a marked constriction above and a marked dilatation below the stimulated area. This phenomenon has been called by Bayliss and Starling ('99 b), the 'law of the intestine.' They state this law as follows: "Local stimulation of the gut produces excitation above and inhibition below the excited spot. These effects are dependent on the activity of the local nervous mechanism."

That Bayliss and Starling ('99 a) exclude absolutely the fundamental arrangement of the two muscular layers as shedding light on the interpretation of the peristaltic wave is seen in the following statement (p. 114): "We cannot imagine any muscle fibre or collection of muscle fibres which would relax on one side and contract on the other side of an excited point."

Bayliss and Starling ('99 d) invoke, therefore, the aid of hypothetical nerve paths to explain the apparent paradox as follows (p. 115): "The different time relations of the two reflex actions would lead one to guess that the system is composed of long paths which conduct inhibitory impulses downwards, and short paths which carry augmentor impulses from one cell station to another in an upward direction." After twenty years no objective histological evidence has been presented confirming the nerve paths assumed by Bayliss and Starling in their interpretation of the peristaltic complex.

Magnus ('04) proved that Meissner's (submucous) plexus is not involved in the production of the slow peristaltic wave. It was modified or inhibited by sectioning the vagus nerve and by the use of nicotine. Consequently, the true peristalsis Magnus considered as due to Auerbach's (myenteric) plexus. Cannon ('12 b) therefore called the 'law of the intestine' the myenteric reflex.

That Mall ('96 c) surmised the active influence of the muscular arrangement in the propulsion of food through the intestine is seen in the following:

"It therefore seems that there are at least two forces which move a body through the intestine. 1. The body as an irritant causes the circular muscles above it to contract, which in turn pushes the body down. A new portion of the mucosa is irritated, which causes a new contraction and so on. 2. At the same time a sucking force, due to an active dilatation below the body, may have a tendency to drag it down."

Mall, however, did not reveal the factor causing the active dilatation. Cannon ('02 c) pointed out this discrepancy in Mall's argument, as follows: "In what manner an active dilatation of the intestinal wall may occur so as to produce a 'sucking force,' Mall does not wholly make clear." At the same time, Cannon also refrained from clearing up the question at issue.

The object of this paper, therefore, is to report results of experiments and of direct observations of the musculature of the small intestine in the light of the spiral architectonics of the two muscle layers. *The law of the intestine or myenteric reflex is proved to be an inevitable duplex contraction phenomenon based on the fact that the inner coat is a close spiral and the outer an elongated spiral or longitudinal muscle layer. This arrangement of the two sets of muscles results in a differential rate of translatory progression of the two contraction waves in the outer and inner coat, respectively. The wave in the outer coat will pursue a greater longitudinal distance per unit of time than that in the inner coat, because the wave in the inner coat pursues a more rotary course, to reach the same destination, than that in the outer coat.*

Experimental observations

A. *Experimental exsection of the inner close spiral muscular coat of the intestine.* The following observations are true for the dog, cat, pig, sheep, and cow. About a foot of the small intestine was inverted under warm oxygenated Ringer's solution soon after the animal was killed. The mucosa and submucosa were

quickly dissected, making an annular gap of about 1 inch. By making two longitudinal incisions, one along the mesenteric and the other opposite the mesenteric attachments through the inner spiral coat, the latter may be quickly dissected from the outer coat. The gut is then reinverted to its normal position (fig. 2). After a quick, clean dissection and if the excitability of the musculature is still present, a satisfactory demonstration may be made (figs. 2 and 3).

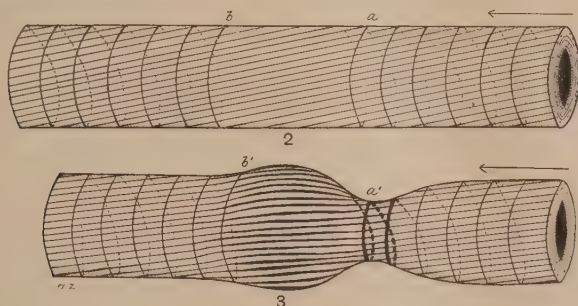


Fig. 2 This represents a segment of the gut from which the inner spiral coat was excised between *a* and *b*. The arrow is directed caudad.

Fig. 3 This represents a peristaltic wave in relation to the area of excision of the inner close spiral muscle coat. *a'* designates the cephalic constriction. From *a'* to *b'* depicts the caudal dilatation. This bulging immediately evident is due to the outer elongated muscle layer. A stimulus applied to the muscle layers of the gut will produce two effects due to the different directions of the muscular fasciculi. The reacting contraction wave of the inner coat will pursue a rotary course, whereas that of the outer coat will pursue a translatory course. Therefore, the cephalic constriction produced by the contraction of the stronger inner coat is bound to trail the caudal dilatation produced by the outer coat.

Stimulating by pinching or by inserting a vaselinated cotton bolus or by means of the induction current, a peristaltic wave may be started above the annular excised area. The dilatation is immediately detected below the stimulated spot and constriction above. It is definitely evident by direct observation that the outer muscle coat contracts in advance of the inner muscle coat as the constriction nears the annular area of excision. The constriction due to the stronger inner coat is cephalad or proximal to the area of excision, at the same time that the outer muscle

coat contracts caudad or distal approximating the edges of the exsected region a' to b'' (fig. 3).

Simultaneous with the approximation of the edges of the exsected area there is a tendency to bulging. This bulges primarily outward, but may bulge inward. This is comparable to the bulge of the fibrils of a twisted string when relieved of the torsion. The fibrils as they unwind tend to give a characteristic spindle-shaped bulging. That a shortening of the outer coat takes place in advance of that of the inner coat is, therefore, objectively evident.

This experiment proves that the active contraction of the outer coat is a definite factor in the production of the caudal dilatation seen in the intestinal peristalsis. The constricted, cephalic component is usually blocked upon reaching the exsected region, in a few instances a slight propagation was detected distal to the exsected region, showing that the elongation of the inner coat produced by the contracting outer coat had taken place. If only a 5- or 10-mm. annular gap is made, the wave is not as frequently blocked as when 30 or 40 mm. are exsected.

The inner and outer coats act, therefore, as reciprocal elongators during the propagation of the peristaltic wave. The integrity of each muscle is necessary for normal peristalsis. Normal peristalsis is a double contraction wave. The wave of contraction of the inner coat, following the path of a close spiral, trails that of the outer coat which follows the path of an elongated spiral. The contraction of the inner coat causes the cephalic constriction, that of the outer coat the caudal dilatation.

B. Experimental exsection of the outer elongated spiral muscle coat. The small intestines of the same animals are used as in the foregoing experiment. The serosa and outer muscular layer are exsected under warm oxygenated Ringer's solution. Two circular incisions of varying distances are made. The incisions are connected by means of two longitudinal incisions, one along the mesenteric and one opposite the mesenteric attachment. The incisions extend to the circular coat. A quick dissection of the serosa and outer muscle layer is now made, leaving the inner muscle layer between the circular incision (fig. 4).

By now stimulating the intestinal tube as before, the typical cephalic constriction and caudal dilatation of the peristaltic wave are started down the tube in the normal direction caudad. In figure 5 the peristalsis is seen a few millimeters from the ex-

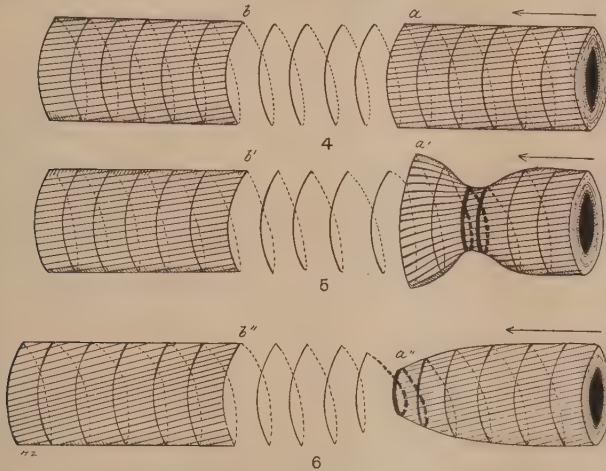


Fig. 4 Diagram of gut from which the outer muscular coat was exsected from *a* to *b*.

Fig. 5 Diagram of peristaltic wave bordering the area in which the outer muscle layer is absent. Note especially that the caudal dilatation is shortened and totally lacking over the area in which the outer muscle layer was exsected.

Fig. 6 The cephalic constriction of the peristaltic wave borders the upper aspect of the area in which the outer muscle layer is absent. From *a''* to *b''* no dilatation is seen. This gap acts as an effective barrier to the propagation of the peristaltic wave if it extends 30 to 40 mm. in length. A greatly diminished wave was seen once to extend caudad in which the gap was only 5 mm. Such a gap ordinarily acts as a barrier if no fluid content is within the lumen of the intestine. At *a''* rhythmic contractions may occur for some time before the gut musculature comes to tonic equilibrium at this point. That the two muscle layers are reciprocal elongators as peristalsis advances is evident.

sected zone with a foreshortened caudal dilatation. As soon as the constriction reaches the edge of the operated region (fig. 6) no caudal dilatation is detected. The outer coat is absent at the gap and coincident with the absence of continuity of the outer coat there is an absence of the typical caudal dilatation.

If the outer coat is exsected for only 5 mm., an abortive continuation of peristalsis may infrequently be detected. But primarily the lack of anatomical integrity of the outer coat acted as an efficient block to the propagation of peristalsis. Frequently a rhythmic series of contractions similar to rhythmic segmentation is detected at the cephalic aspect of the gap.

Interpretation

These two experiments prove that the anatomical continuity of the outer and inner muscular coats is necessary for the conduction of the normal peristaltic wave. When the inner coat is exsected the caudal dilatation is seen over the operated region concomitant with the cephalic constriction a few centimeters above. This proves that the inner stronger muscle is the cause of the cephalic constriction and that the caudal dilatation is due to the outer longitudinal coat.

When the outer longitudinal coat is dissected off, there is a progressive shortening of the caudal dilatation as peristalsis nears the exsected region. As the constriction nears this zone there is a progressive shortening of the caudal dilatation until it is totally absent as the constriction borders the area of operation. This confirms the conclusions reached in the first experiment.

The normal peristaltic wave is an exaggeration of the waves of rhythmic segmentation. The former presents a zone of constriction 6 to 8 cm. long in the dog's gut, whereas the latter presents constricted zones only 1 cm. long. The peristaltic wave or diastalsis has a longer constricted zone, due to the fact that a greater resistance is presented. It is the duplex contraction wave which overcomes the resistance in propelling the contents of the small intestine caudad.

On the other hand, the waves of rhythmic segmentation knead or segment the intestinal contents. A less resistance is presented, consequently a shorter constriction is found. In order that rhythmic segmentation may be effective, about 1 to 2 cm. of intestinal musculature is necessary. What the determinants are that cause either the diastaltic or rhythmic waves of segmentations are not, as yet, determined.

Cannon ('12 d) found that the "normal gastric peristalsis does not require the reflex mechanism for the waves sweep from the pulsatile source to the pylorus, in an orderly manner, after the myenteric plexus has been completely interrupted by a half dozen incisions encircling the stomach." In the small intestine Cannon ('12 e) made encircling incisions through the musculosa to the submucosa, "at intervals varying between 1.5 and 2 cm. throughout the first 45 cm. of the small intestine." He found that segmentation is present in the operated region and that peristalsis is stopped.

The inference, however, that "peristalsis of the small intestine, therefore, unlike that of the stomach, is seriously interfered with by division of the myenteric plexus," Cannon ('12 f) is not to be derived from these experiments. As regards the small intestine, the intervals between the encircling incisions are too narrow for effective muscular waves of normal peristalsis. As Cannon ('12 g) observes in the cat, the trough of the wave is 4 to 5 cm. long. Those encircling incisions have intervals, the longest of which are only 2 cm.; therefore, the musculature has been effectively put out of commission in order to manifest diastalsis, but its integrity is still intact to exhibit the shorter waves of segmentation. I repeated Cannon's experiments on the small intestine of the cat, cutting encircling incisions at intervals of 10 to 15 cm., and found that the normal peristaltic wave was present and had not been interfered with by division of the myenteric plexus.

If it is remembered that diastalsis is the wave of propulsion and that segmentation is the wave of kneading, the difference of resistance to be overcome by each is self-evident. Diastalsis needs a greater extent of the gut for effective muscular action than that of segmentation. If the anatomical continuity of the optimum extent of the gut, necessary for diastalsis is interfered with, it cannot be elicited. The integrity of the muscle was so interfered with in Cannon's experiments for effective peristaltic action.

This action was not inhibited in the stomach, due to a number of circumstances. First, the short length and greater width of the stomach compared with that of the small intestine. Second,

when the musculature of the stomach contracts between the encircling incisions, a greater action is manifested on account of the greater encircling extent of the fibers as compared with the shorter encircling extent of the fibers of the small intestine. Third, the reaction to muscular contraction in the stomach, therefore, is greater. The fluid contents of the stomach, consequently, will act as a medium for the propagation of the distending wave, causing a stretching and subsequent contraction of the fibers between the next neighboring encircling incisions.

In the small intestine the lessened diameter will not allow for as great a muscular action along 2 cm. linear extent as along a corresponding portion of the stomach. A greater extent of anatomical continuity is necessary, therefore, in the small intestine than in the stomach in order to make a similar comparison of muscular action between these two organs. By making the intervals 10 cm. between the encircling incisions in the small intestine, the peristaltic wave is not inhibited.

The difference in the power of the stomach and small intestine is readily apparent by the following brief consideration of the physical principles of the screw: The screw is a modification of the inclined plane, and the conditions of equilibrium are those which obtain in the case of the plane. The resistance, which is either a weight to be raised or a pressure to be exerted, acts in the direction of the vertical, and the power acts parallel to the base; hence we have $P:R = h:2\pi r$; r being the radius of the cylinder and h the pitch of the screw. (The vertical distance between any two threads of a screw measured parallel to the axis is called the *pitch*.)

The power is usually applied to the screw by means of a lever, as in a bookbinder's press. In the gut the power is derived by muscular contraction and the principle of the screw may be stated to be generally, the *power* of the screw is to the *resistance*, in the same ratio as that of the *pitch* of the screw is to the *circumference* of the apparent circle through which the power acts. This means that, other factors being equal, a greater power would be manifested in a given segment of the stomach than in a corresponding length of the small intestine because of the difference in circumference.

Reciprocal elongation of muscles

The contractile state of muscle, as well as the relaxed, arises from a power inherent in itself. The elongation or stretching superimposed on a muscle in tonic contraction depends on some extrinsic power. Simple relaxation of a contracted muscle is not sufficient to enable it to produce another requisite effect. It is necessary that there should be an elongator equal to the quantity of contraction intended to be produced. No muscle has the power of adequately extending or stretching itself; therefore, there must be an elongator. The elongators are usually muscular, but elastic tissue may serve this function as well as fluids in musculo-tubular organs, like the bladder.

The reciprocal elongation of muscles is strikingly evident in the intestine. Rhythmic contraction is due to a reciprocal mechanism, each wave is composed of a contraction and an elongation of the inner spiral coat alternating with a contraction and an elongation of the outer elongated spiral or longitudinal muscle coat. At the start the contraction waves of both coats begin together, but, due to the rotary course of the inner wave and the translatory course of the outer wave, the former and stronger one will inevitably trail the latter and weaker one. The outer and inner muscles are reciprocal elongators, therefore, as peristalsis extends through the intestine (figs. 7 and 8).

When the outer and inner muscles are in normal tonic equilibrium (fig. 7) no distortion is evident. As soon as a contraction wave starts the balance is upset. The stronger cephalic constriction (fig. 8) causes an elongation of the outer muscle coat. The wave of the latter follows in the path of the distal region of elongation. The contraction of the outer coat causes an elongation of the inner coat in the region of the caudal dilatation. Subsequently, the contraction wave of the inner coat is seen to occupy the former zone of stretching in the region of the caudal dilatation. There is, therefore, a definite syncopation in the activity of the outer and inner muscle coats as the peristaltic wave travels through the intestine. The muscle coats act as reciprocal elongators, consequently peristalsis progresses for a variable distance through the gut instead of coming to a dead center.

Direct observation of diastalsis

When the stomach is distended with food, a discharge of chyme into the duodenum takes place. Upon dilatation of the pyloric constrictor there is then produced an elongation of the outer and inner muscular coat overlying the bulged area. The reaction to this stimulus of elongation is contraction. The stronger

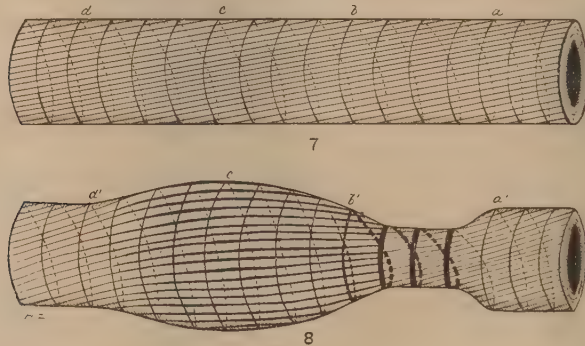


Fig. 7 Diagram of small intestine in which the two spiral muscular layers are in tonic equilibrium.

Fig. 8 Diagram of peristaltic wave. The balance of tonicity between the inner and outer muscle layers is upset. From a' to b' the stronger inner coat upsets the balance by causing the cephalic constriction. In the dog this trough is 6 to 8 cm. long. From b' to d' the outer coat upsets the balance by contracting. This shortens and dilates the gut. The action of the outer muscle layer acts as an effective elongator of the inner layer. This stretching stimulus puts the inner layer in a condition for immediate contraction. The outer muscle layer is put in tension caudad to d' . The contraction wave, therefore, travels progressively down the tube, due to the effective reciprocal stretching of the muscle fibers taking place distal to the contraction wave. The cephalic aspect of the tube is to the right, the caudal aspect to the left of the observer.

inner coat contracts and causes a cephalic constriction. The outer coat contracts and tends to shorten the gut forming the caudal dilatation.

The spiral structure and screw-like action of the intestinal muscles help to explain the normal direction of the peristaltic wave from above downward. Again, the fact that contraction of muscles follows in the wake of a stretching or an elongation

also acts as an effective determinant stimulus. Since the cephalic aspect of the small intestine is the first part stretched by the chyme flowing through the pyloric valve caudad, this region will be the first to respond to the elongating stimulus by contraction.

This muscular action propels the food still further caudad. Successive areas are progressively elongated. They respond in turn by contraction. This alternation of stretching and subsequent contraction of the muscle continues on down the small intestine. The effective stimulus of elongation due to the intestinal contents together with the continuous spiral nature of the musculature readily explains the normal peristalsis as a wave progressing from above downward.

Once this direction of action is implanted in the spiral muscles, it tends always to react by screwing in the same direction as that which the food would normally take. There seems to be a physiological polarity. Every section cut from the gut tends to keep the polarity of cephalad and caudad that it originally possessed while in the intact gut. This is comparable to the analogy cited by Loeb ('12) with regard to the magnet, as follows:

"If a magnet is broken into pieces, every piece has its north pole on that side which in the unbroken magnet was directed toward the north. Likewise, there are animals every piece of which produces, at either end, that organ toward which it was directed in the normal condition. We may speak in such cases of polarization."

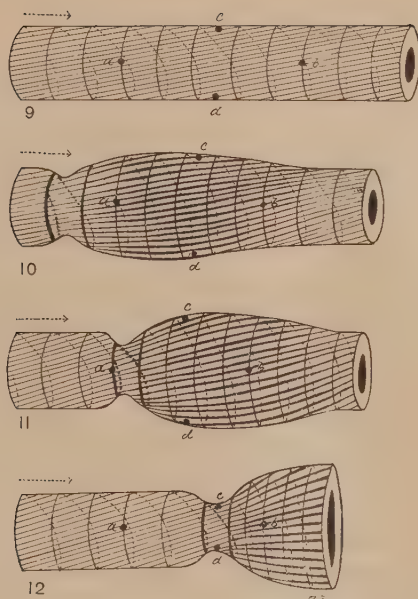
This would explain the stasis observed by Mall ('96 d) in experiment upon the reversal of the gut. The reversed segment by maintaining the original screw-like action that it had in the intact gut would now counteract a wave of peristalsis progressing downward. This is comparable to two screws opposed in action.

There is, however, eventually a tendency for the reversed segment of the gut to become adapted to its abnormal environment. It transmits subsequently the wave of peristalsis from above downward directly opposite to that which it did previously. This reversed conveyance of food was seen by Kelling ('00), Enderlen and Hess ('01), Beer and Eggers ('07), McClure and Derge ('07).

One fact to be emphasized finally in regard to the movement of the intestine is the left-handed screw action of the spiral musculature. A section of the pig's intestine 6 inches in length was exsected under warm saline solution. By inserting a vaselinated cotton bolus in the cephalic opening of the gut, a strong cephalic, anemic constriction is elicited. In a short time the bolus is expelled at the caudal orifice of the intestine. Inspection of the external surface of the bolus reveals a left-handed spiral arrangement of the vaseline coat. The same may be observed on a bolus of warm agar-agar or soft paraffin. The actions of the intestinal spiral muscles are, therefore, in the manner of a left-handed screw in the pig's intestine. These observations were made on the killing floor in the Cudahy abattoir, South Omaha.

The explanation of Bayliss and Starling's graphic record is schematized in figures 9, 10, 11, 12, 16, 17, 18, 19, 20, 21, 22. At each rhythmic contraction of the inner coat a contraction wave is started through the outer muscle coat. To detect the contraction waves in each muscle coat two enterographs are placed at right angles to each other. A shortening of the distance between *a* and *b* (fig. 9) is indicative of contraction in the outer layer of muscle. A shortening of the distance between *c* and *d* (fig. 9) indicates a contraction of the inner muscle coat. As the cephalic constriction progresses caudad toward the registering points, rhythmic contractions of the outer muscular layer corresponding to each contraction of the inner coat are detected in the area of caudal dilatation. This is considered by Bayliss and Starling as due to inhibitory nerve impulses which prevent contraction of the inner muscle. But it is due, however, to the contraction of the outer coat which throws the balance of tonic equilibrium between the two muscle layers in favor of the contracting outer coat, thus causing an active dilatation. This enlargement is not due to a passive relaxation or inhibition of the tonicity of the inner muscle layer. This was definitely proved in the two experiments cited above.

When the zone of cephalic constriction causes a graphic registration of the approximating points *c* and *d*, there is also a shortening between *b* and *a*. Both layers will register simultaneous



Figs. 9 to 12 These figures are explanations, based on the spiral architecture of the intestinal muscles of Bayliss and Starling's graphic record (*Journal of Physiology*, 1899, vol. 24, p. 107). They state that there is synchronous activity at the same spot and at the same time of the two coats (fig. 4, *Journal of Physiology*, 1899, vol. 24, p. 105). Their record was produced by two enterographs placed at right angles to each other. One records the shortening between *c* and *d*. This represents contraction of the inner coat. The other records shortening between *a* and *b*. This represents contraction of the outer coat. The arrows are directed caudad to the right of the observer.

Fig. 9 This figure represents the intestinal musculature in tonic equilibrium.

Fig. 10 This figure represents a peristaltic wave produced by a stimulus. The stimulus may be a pinch, induction current, or a balloon inserted into the lumen as used by Bayliss and Starling. The inhibition of the contraction in the inner coat is due to the elongation caused by the contraction of the outer coat. The so-called inhibition is found in the zone of caudal dilation. Their records prove that the outer coat causes a widening and continues contracting. Each contraction of the outer coat corresponds to the rhythmic contraction of the cephalic constriction as it progresses caudad.

Fig. 11 The cephalic constriction as schematized has passed the upper point *a*.

Fig. 12 This figure represents the cephalic constriction at the points *c* and *d*. There is now produced a record of contraction of the inner coat. At the same time a record is produced of contraction of the outer coat. This is due to the point *a* approximating *b*. The record shows, therefore, simultaneous contractions, but this does not prove that the effects of the contractions occur at simultaneous points. The resultant of contraction of the outer coat is distal to that of the inner coat in the same unit of time. This is inevitable from the arrangement of the muscle fibers and objectively evident by direct and experimental observations.

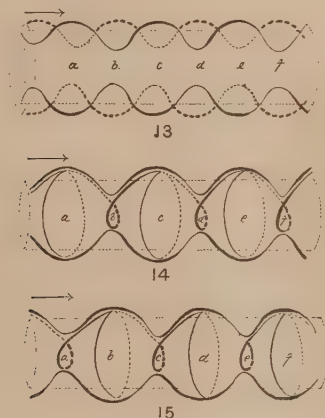
contractions, but the resultant of contraction of the inner coat is a localized cephalic constriction, whereas, in the same unit of time the resultant of contraction of the outer coat is a caudal or distal dilatation. These resultants Bayliss and Starling failed to take into account.

At each rhythmic contraction of the inner close spiral musculature, a simultaneous contraction takes place in the outer muscle layer. The wave of contraction in the outer coat will continue to travel down the intestine. With the next successive and progressive contraction of the inner muscle a second simultaneous wave of contraction is sent through the outer layer. A third, a fourth wave, and so on, are successively started, but the consecutive intervals between the advancing contractions of the outer coat are foreshortened. This leads to a summation of contractions resulting in the caudal dilatation.

This zone of caudal dilatation is produced by manifold contraction waves of the outer coat. Each wave of contraction is immediately preceded by a zone of stretching or tension. Into these elongated areas the trailing contractions take place. The stretching or elongation of the outer is produced by contraction of the inner coat. The latter causes elongation of the intestine and at the same time stretching of the outer muscle coat.

The duplex contraction phenomenon, of a cephalic constriction and caudal dilatation, is due to the structural arrangement of the outer and inner muscular coats of the intestine. A stimulus will start a contraction wave in each simultaneously. The contraction wave traveling in the inner coat in a rotary manner will inevitably trail that of the outer coat pursuing a translatory course. This is comparable to an electric current traveling in a straight wire from *a* to *b* and the same intensity of current traveling in a spirally coiled wire through the same linear distance. It is at once evident that it would take longer for the latter current to reach the same destination on account of the greater distance traveled in a rotary direction as compared to the shorter linear distance traversed by the former.

This analogy may be directly applied to the two contraction waves involved in any type of muscular movement of the in-



Figs. 13 to 15 These figures are diagrams of successive waves of rhythmic contraction. They illustrate the reciprocal elongation of the muscle coats in contraction. Rhythmic contraction is based upon the arrangement of the muscular fasciculi.

Fig. 13 This figure depicts in a reconstruction the alternate contractions and dilatations of two successive rhythmic waves. At the point *a* there is a dilatation simultaneously with contraction at the point *b*. This wave continues on in like manner through the points *c* and *d*, and through the points *e* and *f*. The successive wave is shown by the broken curved lines.

Fig. 14 This figure represents the isolated first wave extending progressively caudad, as depicted in figure 13 as continuous curved lines.

Fig. 15 This figure represents the second or successive isolated wave shown in broken curved lines in figure 13. It is evident that the contraction at the point *a*, figure 15, occurs in the location previously stretched or dilated in figure 14, point *a*. This type of segmentation is produced by successive contraction waves extending through the gut. When the outer muscle coat contracts there is a thickening of the fibers on the proximal side (cephalic aspect) of the area of dilatation. At the same time there is a stretching or elongation of the fibers on the distal side (caudal aspect) of the area of dilatation. This is represented by a thickening and attenuation of the outer lines, respectively. When the outer muscle coat contracts, the resultant shortening and dilatation also elongate the inner muscle coat. Thus, the deformation produced is due to the upsetting of the equilibrium of the two muscle layers by contraction of the outer coat. This stretching stimulus applied to the inner coat results in contraction. The contraction wave progresses into the next stretched zone, and so on down the gut. Rhythmic contraction and diastalsis, therefore, are actions based on the definitive structural plan of the musculature. The effective manifestation of either one or the other of these two actions depends upon the efficient reciprocal elongation of the two sets of intestinal muscles. This is experimentally proved by dissecting off either one or the other of the two layers. An effective block to the propagation of the normal waves is at once established.

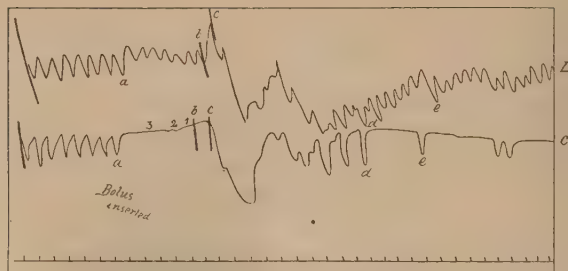


Fig. 16 This figure is taken from Bayliss and Starling's ('99) (*Journal of Physiology*, vol. 24, p. 107, fig. 5) enterographic record of the simultaneous contractions of the outer and inner muscle coats of the intestine. Their interpretation of the record is as follows: "At the beginning of the observation the intestinal wall was contracting rhythmically, the contractions affecting both coats, and being synchronous in both. At (A) a bolus made of cotton-wool coated with vaseline was inserted by an opening into the intestine $4\frac{1}{2}$ inches above the enterographs. It will be seen that the contractions of the circular coat cease instantly and this inhibition is accompanied by a gradually increasing relaxation. There is some relaxation of the longitudinal coat, but the rhythmic contractions do not altogether cease. On inspecting the intestine it was seen that the introduction of the bolus caused the appearance of a strong constriction above it. This constriction passed downwards, driving the bolus in front of it. The numbers above the tracing of the circular fibers indicate the distance of the bolus in inches from the uppermost enterograph lever. At (B) the bolus had arrived at the upper longitudinal lever and at (C) had passed this and was directly under the transverse enterograph, or a little below it. At this point a strong tonic contraction of both coats occurs, expelling the bolus beyond the levers. This strong contraction passes off to be succeeded by another, which like the first is moving down the intestine. In this second tonic wave the rhythmic contractions are evident, superposed on the curve. After the passage of the bolus there is a shortening of the gut (increased tone of longitudinal fibers)."

According to the interpretation of the writer, the 'inhibition' or 'gradually increasing relaxation' seen in the record from *a* to *c*, upon insertion of the bolus above, represents a gradually increasing tension or stretching of both layers as the cephalic constriction nears the contraction record beginning at *C*. Immediately preceding each contraction of the outer layer there is a zone of tension into which each wave of contraction enters. The caudal dilatation, therefore, is a summation of advancing contraction waves closely following one another. This is objectively evident in the curve for the outer layer, *L*, from *a* to *c*, in which rhythmic contractions are taking place. Immediately preceding each contraction is a zone of stretched muscle fibers, the sum of which would tend to lengthen slightly the caudal dilatation over that of the same length of intestinal musculature either in tonic equilibrium or in rhythmic segmentation. The more rapid rhythmic contractions and increased stretching of the outer layer throw the balance of equilibrium between the outer and inner layers in favor of the former. The inner layer in the area of caudal dilatation is held, therefore, in a state of gradually increasing tension or stretching until the optimum at the point *c* is reached. At the point *c* both layers record contraction waves, but it must be remembered

testine. The contraction wave traveling in a rotary manner in the inner close spiral muscle will trail the contraction wave traveling in the outer elongated spiral muscle. The former and stronger contraction will cause a cephalic constriction, the latter and weaker contraction will cause a caudal dilatation (figs. 7 and 8).

The fasciculi of the inner coat make one complete turn in every 0.5 to 1 mm., whereas those of the outer coat make one complete turn in every 200 to 500 mm. Consequently, if we consider the fasciculi as not interconnecting, which they do, however, we will find that a contraction wave that travels 5 mm. in a linear direction through the inner coiled muscle coat will find its complimentary contraction wave that started at the same time and place in the outer coat to have traveled approximately 300 mm. in a translatory direction. This estimate is based on an intestine 25 mm. in diameter (figs. 18, 19, 20, 21). The intestinal movements are rhythmic, however, and this characteristic is due to waves transmitted in each coat at each duplex contraction, thereby causing a reciprocal elongation and mutual coördination of the two waves characterizing peristalsis or segmentation (figs. 13, 14, 15).

that these contraction waves at any period of observation occupy areas of former tension. The tension of the inner coat is caused by the outer one and the tension of the outer coat is caused by the inner one. The double phenomenon of tension prior to contraction travels quickly down the intestine in the outer coat. The relationship of prior tension and trailing contraction is comparable to a gymnast climbing a rope. The tensed rope in advance corresponds to the zone of tension in the intestinal musculature and the progressing gymnast represents the advancing contraction wave.

The graphic record also strikingly shows that the waves travel in a translatory direction much faster in the outer than in the inner muscle layer due to the rotary direction of the path in the latter. The record shows that seven waves in the outer coat have passed the recording point before the inner wave of contraction has reached the point *b*. This fact is also verified after the main rhythmic tonic waves of diastalsis have passed the recording levers at *d* and *e* (*d* and *e* were inserted by the writer). The intestinal muscles are now tending to equilibrium and have not reached as yet the state in which each contraction of the outer and inner coats show the simultaneous contraction curves prior to the insertion of the bolus. *L*, contraction curve of outer fibers. *C*, contraction curve of inner fibers. Contractions recorded by down strokes of enterographic lever, relaxations by upward movement of lever.

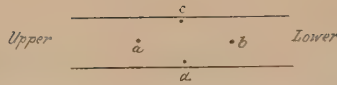
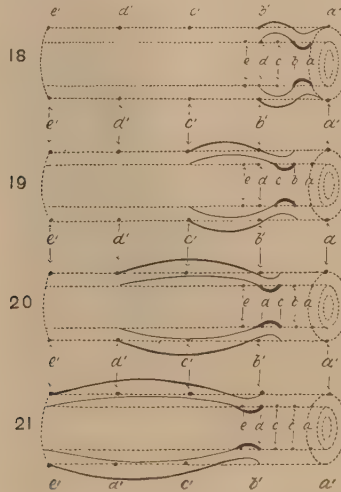


Fig. 17 This figure represents the position of the enterographs which were placed at right angles to one another in Bayliss and Starling's experiment (*Journ. Physiol.*, vol. 24, p. 107, fig. 5a) *a* and *b* are the levers of the enterograph recording the contractions of the inner muscle coat.



Figs. 18 to 21 These figures represent the initial progressively increasing caudal dilatation synchronous with successive contractions of the inner coat cephalad.

Fig. 18 This figure shows contraction of the inner coat from *a* to *b* simultaneous with the contraction of the outer coat through one-half the distance from *a'* to *b'*. The contraction wave of the outer and inner coats are preceded by zones of tension or stretching of the muscle fibers produced by the reciprocal elongating actions of the two muscle layers.

Fig. 19 This figure represents the contractive wave of the inner coat advancing from *b* to *c* synchronous with an increasing caudal dilatation produced by the outer coat midway between *a'*, *b'* to *c'*.

Fig. 20 This figure depicts the caudal dilatation still further advanced from *b'* to *d'* at the same time that the inner constriction travels from *c* to *d*.

Fig. 21 This figure demonstrates the advancing contraction wave of the inner coat occupying the distance from *d* to *e*. The caudal dilatation extends from a point midway between *b'*, *c'* to *e'*. With each successive rhythmic constriction produced by the cephalic contraction wave in the inner muscle fibers a corresponding wave is initiated in the outer muscle layer. The summation of the contraction waves in the outer group of fibers produces the caudal dilatation due to the greater activity of these fibers over that in the inner layer. The balance or equilibrium is upset, therefore, in favor of the outer fibers at this point.

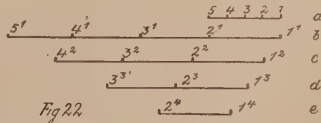


Fig. 22 This figure is a diagram representing the differential rates of transitory advance of the successive contraction waves in the outer and inner layers of muscle fibers. Line *a* represents the inner close spiral muscle fibers. Lines *b*, *c*, *d*, and *e*, the outer elongated spiral muscle layer. Upon stimulation there is produced a rapid caudal dilatation and a slowly produced cephalic constriction. Contraction waves begin simultaneously, in both coats, but, due to the linear course followed by that in the outer muscle layer, a difference in time takes place before the effects of contraction are objectively evident in the two layers of muscles. When the cephalic constriction advances from 1 to 2 line *a*, the caudal dilatation is evident from 1' to 2' line *b*. When the cephalic constriction progresses still further from 2 to 3 line *a*, the caudal dilatation is a summation of two contraction waves in the outer fibers from 2' to 3' line *b*, and 1'' to 2'' line *c*. Likewise with continuation of the constriction produced by the inner coat from 4 to 5 line *a*, the caudal dilatation is a summation of four consecutive waves closely following one another, namely, 4' to 5' line *b*, 3'' to 4'' line *c*, 2''' to 3''' line *d*, and 1'''' to 2'''' line *e*. From this it is evident that the degree of caudal dilatation is directly proportional to the extent of the cephalic constriction. If the cephalic constriction is a long trough of 5 cm. or more, the summation of caudal constrictions in the outer fibers is greater than if only 1 cm. is involved in the formation of the cephalic constriction. This explains the difference of extent of the caudal dilatation manifested in the intestinal movement of segmentation and diastalsis.

Conclusions

The Screw-like Action of the Small Intestine

1. Because of the left-handed, helicoidal arrangement of the musculature of the intestine, the intestinal movements are comparable to the action of a left-handed screw.

2. The fasciculi of the inner coat make one complete turn in every 0.5 to 1 mm., whereas those of the outer coat make one complete turn in every 200 to 500 mm. Consequently, if we consider the fasciculi as not interconnecting, which they do, however, we will find that a contraction wave that travels 5 mm. in a linear direction through the inner coiled muscle coat will find its complimentary contraction wave that started at the same time and place in the outer coat to have traveled ap-

proximately 300 mm. in a translatory direction. This estimate is based on an intestine 25 mm. in diameter.

3. *The inner muscular layer is wound as a close spiral. The outer as an open spiral. The difference in rate of translatory progression of the two contraction waves depends upon this muscular arrangement. The wave traveling in the inner group of fibers takes a rotary course, whereas that in the outer fibers takes a more translatory course to reach a corresponding destination. Therefore, the contraction of the stronger inner muscle coat will inevitably trail that of the outer. The arrangement of the intestinal muscle layers clearly explains the phenomenon of cephalic constriction and caudal dilatation of diastalsis without invoking the aid of hypothetical nerve paths. Peristalsis, therefore, is a duplex contraction phenomenon produced by the differential rate of translatory advance of the two contraction waves in the outer and inner muscle layers, respectively.*

4. By exsection of the inner muscular layer of the intestine, it is proved that the caudal dilatation of diastalsis is produced primarily by the summation of contractions in the outer layer of muscle fibers.

5. By exsection of the outer muscular layer, it is proved that the cephalic constriction is caused by the inner layer of close spiral fibers.

6. The movements of the intestine depend upon the reciprocal elongating actions of the outer and inner layers of muscle fibers, respectively. These movements are superimposed on the tonic condition of equilibrium of the two muscle layers. The application of any drug, therefore, that decreases the excitability of the musculature or destroys the tonic equilibrium of the two layers will indirectly effect the typical muscular responses.

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Resumen por el autor, Naohide Yatsu.

Sobre los cambios de los órganos reproductores en la parabiosis heterosexual de ratas albinas.

En las parabiosis de macho y hembra (individuos unidos mediante una operación quirúrgica), algunos de los folículos de Graaf se desarrollan normalmente y forman cuerpos amarillos, mientras que la mayoría experimenta cambios regresivos. En estas parabiosis el útero no se modifica de modo marcado, aun cuando puede presentarse una hiperplasia de la subserosa. Los folículos ováricos de las parabiosis de macho y hembra castrado no se desarrollan normalmente, ni tampoco se forman cuerpos amarillos. Los quistes folículos y los cuerpos atréticos son abundantes. Hay un aumento aparente de las células intersticiales, entre las cuales existen unas cuantas células luteínicas. El útero es el órgano más afectado mediante la unión con un macho castrado. Especialmente clara es la producción de hidrometrias. El testículo y la próstata no son afectados por la unión con hembras normales o estériles.

Translation by José F. Nonidez
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ON THE CHANGES IN THE REPRODUCTIVE ORGANS IN HETEROSEXUAL PARABIOSIS OF ALBINO RATS¹

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SEVEN FIGURES

The actions of products from various endocrine organs have been studied of late so extensively that one can hardly keep in touch with the literature relating to even a single organ. Of the methods employed for such investigations parabiosis has not so far been used as it ought to be. And, I think, much new light will be thrown in the future upon endocrinology by making use of this method. .

In the fall of 1916, several series of experiments in joining together two albino rats of different sexes were begun at the Zoological Institute of the Imperial University, with the help of Mr. Masanosuke Takesita.² The object of these experiments was to obtain evidence regarding the effects of male and female hormones in mixed condition upon united individuals. The present investigation was more immediately suggested by Lillie's paper on the theory of the free-martin which appeared in "Science" ('16). In this paper he expresses the view that in the heterosexual twins of cattle the reproductive organs of the female fetus are impaired by the influence of sexual hormones from the male fetus, provided a blood circulation has been established

¹ In carrying out the experiments described in this paper, I am indebted to the Tokugawa Memorial Fund.

² Mr. Takesita's death took place rather suddenly in November of 1919, just after the operative part of the present investigation came to an end. He graduated from the Imperial University in 1916. At the time of his death he was thirty years old. I owe a great deal to his skill and intelligence in carrying out this study.

between the two. I thought it would be interesting to see how the male hormones act upon the female and vice versa in adult heterosexual parabiosis.

As to the method of parabiotic operation I am indebted not a little to Dr. Rikurô Matsuyama. His kindly advice was invaluable. When I began this work he was engaged in a study of uraemia produced by extirpating the kidneys from one component of rat parabioses. Later, however, he directed his attention to the line which I was following. He obtained results almost the same as mine, and published an excellent paper in Japanese in August, 1919. Yet, since his paper is written in a language only slightly intelligible to our confrères outside this country and because of the existence of discrepancies in our work and of additional observations, the publication of the present paper may not be regarded as superfluous.

Most of the rats used for the experiments were bred in the laboratory. Their ages, body length and body weight were noted at the time of operation (parabiosis and gonadectomy) and when killed. All the parabiotic pairs from which the material was taken had been healthy. It is, therefore, almost certain that any alteration found in the preparations is due to the action of mixed hormones produced by united individuals.

MALE-FEMALE PARABIOSES

I have twenty-four successful cases of male-female parabioses. The period of union ranges from 11 to 179 days. It need hardly be mentioned that the actual parabiotic period does not correspond with that of union for the blood circulation becomes established between two animals only after at least ten days. The youngest animal operated on was twenty-nine days old and the oldest ninety days old. The individuals used for parabiosis were in some cases from the same litter and in others from different litters, but in all cases they were of the same age.

1. Changes in the ovary

The ovaries of parabiotic pairs were cut into sections and compared with those of unoperated females at corresponding ages, which had been kept separated from the males.

At the outset it might be mentioned that the time of appearance of the first corpus luteum, that is, the first ovulation comes at about the same time in the ordinary females and in the parabiotic females. Ovulation may continue for a considerable length of time, as is indicated in a case (161-day-old female, 111 days in parabiotic union) in which several apparently normal eggs at the metaphase of the second maturation mitosis were found in the oviduct. And this is also substantiated by the fact that both Morpurgo ('08) and Matsuyama '19) obtained normally developed fetuses from parabiotic females.

While the formation of the corpora lutea is taking place on the one hand, a great many graafian follicles are found undergoing regressive changes on the other. In fact, many more follicles degenerate in the ovaries of parabiotic females than in ordinary females. This I do not hesitate in attributing to the influence of the male component. Predominance of degenerating follicles makes itself evident after thirty days' union with the male. The longer the parabiotic period, the larger the number of degenerating follicles. It may also be added that the regressive changes set in more in younger follicles than older ones.

There are several modes of degeneration of the follicles. But it is important to note that none of the modes is peculiar to parabiosis. The only difference found between those in the normal ovary and in that of parabioses is in degree of change.

The first indication of degeneration of the follicle is the dissolution of the cells composing the cumulus oöphorus. First the egg is set free in the follicular cavity. Then it is divided or rather fragmented into five or six cells of different sizes in the manner described by Hennuguy ('94) in the rat and by Spuler ('01) in the guinea-pig. Sometimes there are two nuclei in one cell. These 'blastomeres' disintegrate in the follicular fluid so completely that no trace of them can be found. The above changes also take place in very young follicles.

Pari passu with the dissolution of the egg goes the disintegration of the stratum granulosum. This is accomplished in two ways. The first mode is the gradual dissolution of the component cells from the inside, reducing the layer to a very thin sheet of cells, often separated from the theca interna by a narrow space. In an advanced stage, this layer is fenestrated, appearing in sections as an interrupted ring. The other means of destruction is by phagocytes that have entered the follicle from without. Whence they come I am not able to ascertain. At any rate, the phagocytes are much larger than the granulosa cells and

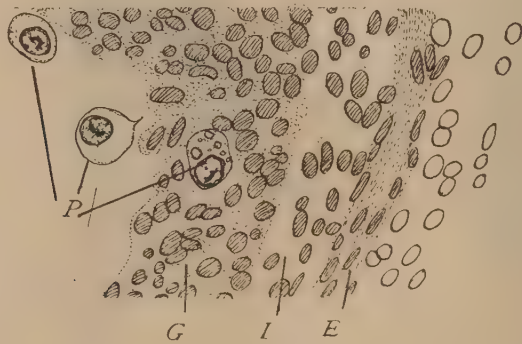


Fig. 1 Portion of a degenerating follicle (forty-seven days of parabiotic union). *P.*, phagocytes; *G.-st.*, granulosa; *I.*, theca interna; *E.*, theca externa. $\times 560$.

are readily detected by ingested debris. They migrate toward the follicular cavity until they occupy a position in the fluid, as is shown in figure 1. As to the fate of these cells, I am unable to offer any suggestion. It is very probable that they also disintegrate in the follicular fluid.

As the granulosa layer disappears, the theca interna increases in thickness. Sometimes this process goes on only in a restricted area, sometimes along the entire layer. In either case the cells constituting the theca interna increase in size. These cells however, are quite different from the lutein cells. The nucleus and the cell body of the former are much smaller, more granular, and stain more deeply with haematoxylin than those of the latter.

The cytoplasm of the lutein cells is more eosinophilic. In cases where the central cavity is obliterated, a corpus atreticum composed of modified theca interna cells is formed.

Side by side with the above-described atretic follicles are found those with the theca cells modified into the lutein cells. Such follicles have often a large central cavity. Matsuyama thinks that this type is peculiar to the ovaries of parabioses, but in my sections of the ovaries of old females this kind of follicle is not uncommon.

A feature that may rightly be called characteristic of the ovaries of parabiosis is the enormous growth of the interstitial cells.

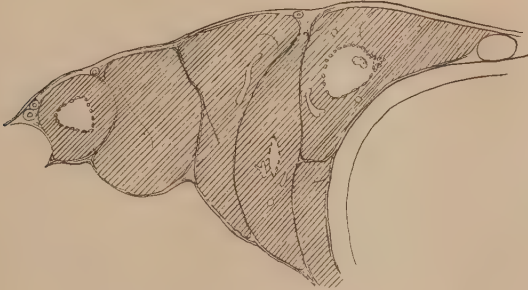


Fig. 2 Portion of ovary showing six groups of interstitial cells (forty-seven days of parabiotic union). $\times 86$.

Matsuyama has noticed this peculiar change also. This is met with especially in the female, which is operated upon when young; that is, at a stage in which the follicles have not yet attained their full size.

It cannot be doubted that the interstitial cells are modified theca cells. Consequently, the follicular cavity is found, in most cases, inside the mass of these cells and in some cases degenerating ova and débris of the granulosa layer, as is shown in figure 2.

The lutein cells are sometimes found among the interstitial cells. They can readily be distinguished, since the former have large nuclei and a single nucleolus in each, while the latter

have small nuclei, with scattered chromatin granules. Is the formation of lutein cells due to immigration or transformation? I think both processes may go on, judging from the fact that few mitotic figures are met with.

In conclusion it may be said that in parabiosis the male exerts a certain deteriorating effect upon a good many graafian follicles of various stages, though some of the follicles remain apparently normal and are able to discharge fecundable eggs. The injurious effect of the male hormones does not produce anything peculiar to parabiosis, but accelerates degeneration processes that would take place in normal ovaries.

2. Changes in the uterus

The changes in the uterus of the parabiotic female are usually not so marked as those in the ovary. As a matter of fact, changes, if any, are so slight that they do not at all affect the normal development of the fetuses, as was shown by the cases of Morpurgo and Matsuyama.

In some females, however, changes are noticeable after a month of parabiotic union. The hyperplasia of the stratum subserosum is the main feature. The uterine glands decrease in number. The muscular layers become thinner. The eosinophile leucocytes seem to be more abundant than in the normal uterus.

It is interesting to note that in two cases the uteri have been modified exactly like those of female + castrated male parabioses, which will be described in the next section. In the two cases the testes were smaller than in other males of parabiotic union, though spermatogenesis was going on normally.³

³ In one of the two males (139 days old when killed, 93 days of parabiotic union, body weight 160 grams, body length 182 mm.) the right testis was 0.977 gram and the left 0.967. In the other male (159 days old when killed, 106 days of parabiotic union, body weight 195 grams, body length 170 mm.) the right testis was 0.338 gram and the left 0.310 gram. In comparison it may be stated that in the males of similar age and of similar length of parabiotic period the testes weigh between 1.100 and 1.200 grams.

FEMALE + CASTRATED MALE PARABIOSES⁴

Males were castrated, and after various intervals they were united with females. I have fourteen successful cases of this kind of parabioses. The period of union ranges from 18 to 179 days.

Contrary to what one might expect, the changes in the ovary and the uterus are more marked in these cases than in the above-described female+uncastrated male parabioses.

1. Changes in the ovary

The ovary is so affected by the influence of the castrated male that it ceases to discharge the eggs, judging from the fact that no normal corpora lutea are formed and what look like them are nothing more than the corpora atretica. All the follicles undergo regressive changes of one kind or another. The processes of degeneration are different in follicles of different stages of development. They are the same as those described by Böshagen ('04), Benthin ('11), Cohn ('09), and others. It may here be mentioned that none of the regressive changes is peculiar to this kind of parabiosis, all being met with in the normal ovary, as is the case with male-female parabioses. But all the modifications come in intensified form.

Of several modes of change of the follicles, I may mention first of all a most striking one, which I would not hesitate to regard as characteristic of this type of parabiosis. This is cyst formation due to enormous growth of the stratum granulosum. One example is shown in figure 3. Here one sees uneven growth of this layer. As the follicular fluid accumulates the follicle is distended, reducing the wall to extreme thinness. Sometimes the blood-vessels make their way into the cumulus oöphorus. The egg in the follicular cysts usually undergoes degeneration without fragmenting. It may occasionally divide, but as far as I know it does not act as in younger follicles.

⁴Harms ('11) has described a case of this kind of parabiosis in *Rana temporaria*, but I am not able to learn from his paper how the female organs were affected by the castrated male.

This remarkable growth of the granulosum layer takes place not long after parabiotic operation. Indeed, I have a case in which the ovary showed this change only eighteen days after union.

At one portion of the follicular cyst one often sees an accumulation of the lutein cells formed from the theca interna. I have no strong evidence to oppose the view that this represents a stage on the way to formation of a corpus atreticum. But I am rather inclined to believe that any follicle once distended on the way to become cystic, will remain as such and never transform into the solid corpus atreticum. As a matter of fact lutein cells are found in the wall of half-grown follicles. This is represented in figure 4. Here the lutein cells are formed not only from the

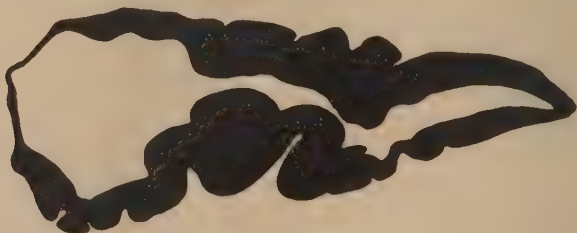


Fig. 3 Stratum granulosum of a follicle (thirty days of parabiotic union).
× 86.

theca interna directly, but also from the interstitial cells. After the disintegration of the granulosum cells of such follicles they turn into solid corpora atretica though sometimes a small cavity appears in them.

Other changes, such as unusual growth of the interstitial cells, are exactly the same as in male-female parabioses.

2. Changes in the uterus

The uterus, of all the female organs I have examined, is the most affected by the union with a castrated male. The striking change makes itself evident as early as eighteen days after parabiotic operation. The normal structure of the uterus will not here be described, since that has been so fully studied by Po-

wierza ('12) in the mouse and by Beiling ('06) in the rat. The first indication of the change is the rapid growth of the mucous layer. This is soon followed by the accumulation of turbid fluid in the uterine cavity. The subserosa or vascular layer, with not a few eosinophils, becomes thinner. The uterine glands may remain for some time compressed within the now narrow

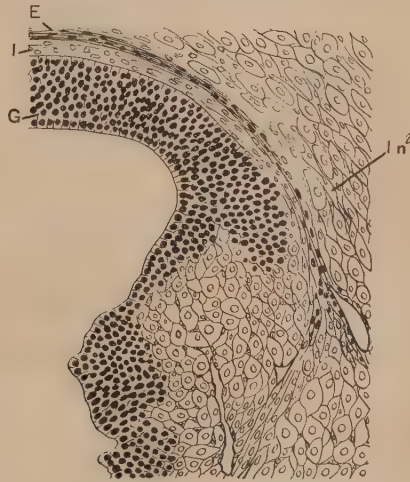


Fig. 4 Part of a follicle showing invasion of lutein cells into st. granulosum (*G.*) from interstitial cells (*In.*) (107 days of parabiotic union). *I.*, theca interna; *E.*, theca externa. $\times 186$.

subserosa. Finally they disappear completely. The longitudinal muscles are no longer found. The diameter of this distended uterus is 6 mm., while that of the normal one is not more than $1\frac{1}{2}$ mm. The wall is reduced to one-tenth the normal thickness. This hydrometral condition is not accompanied by hydrosalpinx, which Fischel obtained experimentally ('14). No noticeable changes take place in the tubal part.

TESTES OF PARABIOSES

The testes of male-female parabioses (twenty-four cases from 11 to 179 days) and of male+spayed female parabioses (eleven cases from 6 to 148 days) were weighed and cut into sections.

It is interesting to note that the histologic structure of the testes is not affected at all by the union with either unoperated

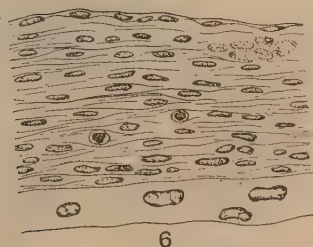
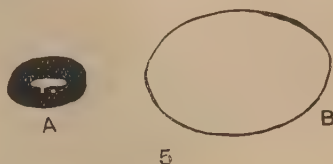


Fig. 5 Section of normal (A) and distended uterus (B). $\times 4.5$.

Fig. 6 Portion of distended uterus (eighteen days of parabiotic union). Notice the remnant of longitudinal muscle fibers. $\times 420$.

Fig. 7 Portion of half-distended uterus (twenty-three days of parabiotic union). Here one notices a uterine gland to the right. $\times 97$.

or spayed female. In the latter combination, however, the sperm formation is somewhat delayed. In one case the spermatozoa could not be seen in a ninety-eight day rat joined for thirty-two days with a spayed female.

RESULTS

That the spermatozoa are functional in male-female parabioses is shown by the fact that a female which had been kept with a male-female parabioses gave birth to a litter. Copulation took place in this case 134 to 135 days after parabiotic operation.

In passing it may be mentioned that the prostate is not affected at all by the union with either normal or spayed female. In castrated male-female parabioses the prostate atrophies as in the solitary castrated male.

1. In male-female parabioses some graafian follicles undergo the normal course of growth and the corpora lutea are formed, while a large majority of follicles undergo regressive changes. None of the changes is peculiar to this kind of parabiosis.

2. In male-female parabioses the uterus is not modified very markedly. Sometimes hyperplasia of the subserosa is noticed.

3. In the ovary of castrated male-female parabioses none of the follicles develops normally. No corpora lutea are formed. Follicular cysts and corpora atretica are abundantly produced. Noticeable growth of the interstitial cells takes place and sometimes the lutein cells are met with in the interstitial cell groups.

4. The uterus is most affected by the union with a castrated male. Hydrometra of various grades is the remarkable feature. The uterine tubes are normal.

5. The testis is not affected at all by the union with either normal or spayed females. The same is true of the prostate.

COMMENT

From the above-described parabiotic experiments rather unexpected results were obtained. One would naturally suppose that the female organs would be more affected by the male with the testes. But as a matter of fact they are more influenced

by the castrated male. To account for this phenomenon I think it is very probable that the endocrine organs of the male are affected by castration, and that the ovary and uterus of united individuals are in turn influenced by the hormone or hormones produced from these organs. But what organ partakes in this process and how I do not know.

It is also interesting to note that the testis is not impaired in the least by the ovary of the female to which it is united.

Anatomical Institute, Keio University,
July 22, 1920

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Resumen por el autor, Howard Homer Bell.

Divertículos del duodeno. Descripción de tres observaciones.

El primer caso estudiado presentaba un divertículo en la región de la ampolla de Vater. El segundo presentaba un divertículo en la región de la ampolla mencionada y otro más inferior en la segunda parte del duodeno; ambos pacientes eran varones y marcadamente obesos. Su edad era cuarenta y dos, y sesenta y un años, respectivamente. El tercer caso presentaba tres divertículos, uno en la papila mayor, otro en la menor y un tercero en la unión de la segunda y tercera parte del duodeno. El paciente era una hembra emaciada de setenta y cinco años de edad. Divertículos semejantes se presentan también en el colon. En todos los casos estudiados la muscularis terminaba bruscamente sin formar parte del saco. Estos divertículos se consideran como adquiridos a consecuencia de la existencia de puntos débiles en la muscularis.

Translation by José F. Nonidez
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DIVERTICULA OF THE DUODENUM

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TWO FIGURES

Diverticula have been observed in all divisions of the gastrointestinal tract. Particular attention, however, is given to diverticula of the duodenum on account of their relation to the common bile and pancreatic ducts and associated organs.

Diverticula of the alimentary tract occur most frequently in the colon, ileum, oesophagus, pharynx, duodenum, and stomach,—in the order named (Buschi).¹ Baldwin² observed fifteen cases of duodenal diverticulum in 105 cadavers. Schüppel (quoted by Baldwin) found seven instances in forty-five bodies, and later at Kiel found one in 200 bodies. Linsmayer³ observed forty-five cases in a study of 1367 autopsies.

Davis⁴ found from a study of the literature that 16 per cent of duodenal diverticula occurred in subjects under fifty years, 37 per cent between fifty and sixty years, and 47 per cent above sixty years. In the series studied by Linsmayer, the youngest showing diverticulum was thirty-six years and another thirty-eight years old. Three instances occurred between forty and forty-nine years, 5 between fifty and fifty-nine years, 13 between sixty and sixty-nine years, 14 between seventy and seventy-nine years and 4 between eighty and eighty-seven years. Wilkie⁵

¹ Virchow's Arch. f. path. Anat., 1911 (206), 121.

² Anat. Rec., vol. 5, 1911, p. 121.

³ Verhandl. d. deutsch. path. gesellsch. 17, 445, 1914.

⁴ Tr. Chicago Path. Soc., vol. 9, 1913, p. 1.

⁵ Edinburgh Med. Jour., vol. 11, 1913, p. 219.

found that in twenty-six cases, seventeen were in males and nine were in females. He believes that diverticula of the duodenum are congenital in origin and bases this belief upon the following data:

1. "The duodenum in the course of normal development gives off hepatic and pancreatic buds; consequently developmental anomalies might be expected to occur more frequently in this than in other regions of the intestinal tract. Tandler ('02) found that congenital atresia occurs in the duodenum 39.6 times as frequently as in any other segment of small intestine of equal length.

2. "The observation of Lewis and Thyng ('08) showed that during early foetal life, diverticula are often met with in the the upper reaches of the small intestines, particularly in the duodenum.

3. "An accessory pancreas is occasionally observed in or on the wall of such diverticulum.

4. "The case reported by Shaw, where in a newborn infant there were found both a diverticulum and a congenital occlusion of duodenum.

5. "In the cases recorded by Letulle ('98) and Falconer ('07) congenital diverticula of the oesophagus and stomach, respectively, accompanied the duodenal diverticula.

6. "Many of these diverticula abut on and even indent the head of the pancreas."

More than 400 diverticula were observed in the gastro-intestinal tract of an adult male (Hansemann⁶). These were situated along the line of attachment of the mesentery corresponding to the points of penetration of the larger vessels, particularly the veins. This relationship of vessels to diverticula has not infrequently been observed. Relaxation of venous sheaths in association with circulatory stasis (Graser⁷), fatty degeneration of the muscularis, (Roth⁸), and fatty infiltration of the intestinal

⁶ Virchow's Arch. f. path. Anat., 1896, Bd. 144, S. 400.

⁷ München. med. Wchnschr., No. 22, 1899, S. 721.

⁸ Virchow's Arch. f. path. Anat., 1872, Bd. 56, S. 197.

wall (Aschoff⁹) were conspicuous in certain cases and considered as the causes of the diverticula.

Baldwin found from the literature that the muscularis was observed in the walls of these diverticula in twenty instances, while in sixteen it was absent. In forty-seven cases there was no report on this subject. (Keith¹⁰) states that it is not uncommon to find in old people in the posterior wall of the duodenum near the termination of the common bile duct diverticula which are pouches of mucous membrane extruded at weak points in the musculature. However, he cites the cases described by Clogg and Thompson as instances of developmental diverticula; these occurred in the small intestine below the duodenum in association with accessory pancreas, and possessed the muscular coats of the intestine.

The arrangement of the muscularis is subject to great variation and aids very little in a study of the etiology of these diverticula. Meckel's diverticulum possesses the musculature of the intestinal tract. Its origin is peculiar and suggests no explanation of diverticula elsewhere.

In most instances diverticula of the duodenum were found at necropsy and had no direct relation with the cause of death. However, in a case reported by Bauer,¹¹ biliary stasis with jaundice was caused by inflammation of a diverticulum at the site of the ampulla of Vater. Death followed intrathoracic hemorrhage. Furthermore, several instances of duodenal diverticula occurred in association with pain in the upper abdomen and disturbances of digestion; either duodenal ulcer or gall-bladder disease was found in this group of cases, which possibly accounted for the symptoms present. However, disturbance of digestion, poor nutrition, and certain nervous manifestations have occurred in association with duodenal diverticula, which were found by x-ray examination or at operation, symptoms that could not be

⁹ Pathologische Anatomie, dritte Auflage, zweites Band, S. 824. Jena, Gustav Fischer, 1903.

¹⁰ Brit. Med. Jour., 1910, p. 376.

¹¹ Wien, klin. Wchnschr., 1912, (25) 879.

explained otherwise than by the diverticula. A diverticulum has led to surgical intervention or has been found at operation and corrected in several instances. Consideration of the facts presented in the literature indicates that the significance of the associated symptoms is uncertain.

I have examined two specimens of diverticulum of the duodenum. The first instance occurred in a teamster, forty-two years old, who related no symptoms indicating that this diverticulum had given him any trouble. He was very obese. Death followed failure of cardiac compensation associated with aortic stenosis and chronic fibrous myocarditis. The anatomical diagnosis made at necropsy was as follows:

Aortic stenosis and insufficiency; hypertrophy and dilatation of heart; recent and old infarcts of lung; chronic passive congestion of all organs; chronic tuberculous process of lung and spleen; nephrolithiasis; obesity; diverticulum of duodenum.

The diverticulum occurred as a blind pocket immediately above and anterior to the major papilla. It communicated with the duodenum through a puckered opening measuring 0.5 cm. in diameter. It was about 2 cm. deep and 1 cm. wide. It extended to the left and slightly upward anterior to the common bile and pancreatic ducts in the fissure between the lobes of the head of the pancreas. The common bile duct was slightly dilated and opened independently into the duodenum beside the pancreatic duct, being separated from it by a very narrow septum. The sac was formed by thinned intestinal wall. No vessels were found which penetrated the sac. The situation of this diverticulum is shown in figure 1.

Sections were made from the wall of the diverticulum. The muscularis ended fairly abruptly and did not form a part of the sac. Considerable fat was deposited in the intestinal wall and some occurred in the muscularis.

The second instance occurred in a man sixty-one years old and showed two diverticula. No symptoms referable to the diverticula were recorded. The patient had been employed in an organ factory. He was very obese and suffered from a long-standing irreducible inguinal hernia. The hernia was

reduced and repaired by operation. The caecum, which was greatly dilated and distended, filled the sac. The patient died a few days following the operation, from peritonitis associated with many ulcers and numerous perforations of the caecum, ascending and transverse colon. The anatomical diagnosis made at necropsy was as follows:

Operative incisions in right thigh and inguinal regions; repair of hernia with fascia transplantation; peritonitis following intestinal ulceration and perforation; follicular enteritis; chronic fibrous myocarditis; arterial sclerosis; focal sclerosis of endo-

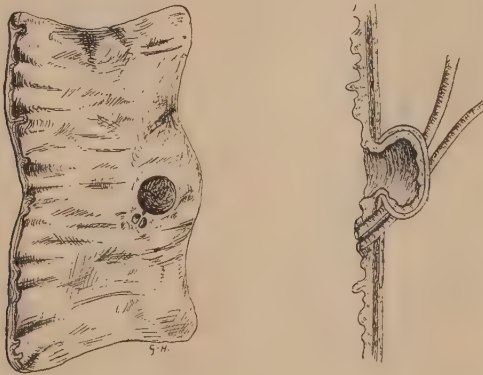


Fig. 1 Drawing showing the inside and lateral views of the duodenum and the relation of the diverticulum to the openings of the common bile and pancreatic ducts.

cardium, aortic and mitral valves; chronic diffuse nephritis with atrophy of cortex and fatty degeneration of pyramids; chronic passive congestion of viscera; chronic bronchitis; fatty degeneration of liver and kidneys; chronic obliterative appendicitis, prostatic hypertrophy; healed tuberculosis of peribronchial lymph nodes; old pleural adhesions; obesity; lipomatosis of pancreas with fat necrosis; diverticula of duodenum; patent foramen oval; accessory spleen.

The first diverticulum occurred at the site of the ampulla of Vater. It communicated with the duodenum through an oval opening measuring about 1 cm. vertically and 0.6 cm. horizontally.

It was about 2 cm. deep and terminated as a blind pocket. The diverticulum extended to the left and slightly upward in front of the common bile and pancreatic ducts in the fissure separating the two lobes of the head of the pancreas. The common bile duct was moderately dilated and opened into the posterior and lower part of the diverticulum, about 0.5 cm. from the intestinal wall, in conjunction with the pancreatic duct. The sac was formed throughout by thinned intestinal wall.

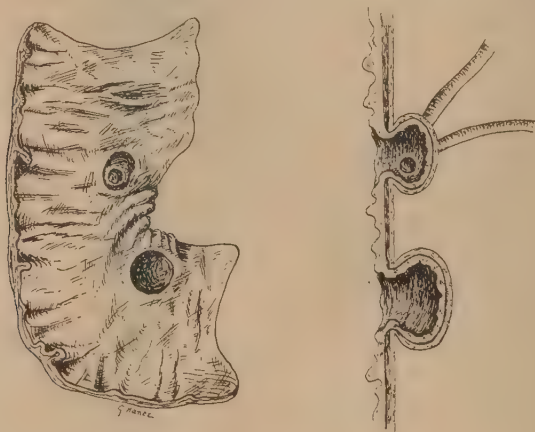


Fig. 2 Drawing showing the inside and lateral views of the duodenum and the relation of the diverticula to the duodenum and the common bile and pancreatic ducts.

The second and larger diverticulum occurred in the lowest part of the duodenum along the posterior surface and rested chiefly upon the vena cava. It was disc shaped, being flattened anteroposteriorly. It communicated with the duodenum through an opening 0.9 cm. in diameter. After fixation the sac measured 3 cm. in its longest dimension and 1.5 cm. in its shortest. It was about 2 cm. deep. The sac was formed by thinned intestinal wall. The anatomical relations of these diverticula are shown in figure 2.

Sections from the walls of the diverticula show that the muscularis ended rather abruptly and did not form a part of the sac.

Considerable fat was deposited in the intestinal wall and some occurred in the muscularis.

The two cases herein reported show some of the conditions frequently observed in association with duodenal diverticula. The patients were male and moderately advanced in life, i.e., forty-two and fifty-one years old, respectively. Each showed much venous stasis. Obesity was marked in both instances. The muscularis ended rather abruptly about the openings and did not form a part of the walls of these diverticula.

A consideration of the facts presented in the literature and of the cases reported here leads one to the conclusion that duodenal diverticula of this type are not congenital, but acquired, occurring at points in the muscularis which are weakened by the passage of ducts and blood-vessels or by pathological processes, and that increasing age and the greater muscular activity in men are factors in their production.

SUPPLEMENTARY REPORT

I have recently studied a specimen, with three diverticula of the duodenum, removed at autopsy.

The patient was a woman seventy-five years old, and was markedly emaciated. No symptoms referable to the diverticula were recorded. There was general arterio-sclerosis with gangrene of the feet. Death followed acute parotitis and *micrococcus aureus* was grown from the parotid gland and the heart's blood.

The anatomical diagnosis was as follows: General arterio-sclerosis; gangrene of the feet; acute parotitis; volvulus of the caecum and transverse colon; carcinoma of cervix uteri with extension and metastasis; chronic nephritis with small contracted kidney; healed calcified tubercles in the lungs, bronchial lymph nodes and spleen; diverticula of the duodenum and colon; chronic interstitial pancreatitis; periportal cirrhosis of the liver.

The largest diverticulum occurred at the junction of the second with the third part of the duodenum, along the upper border, 22 mm. below the major papilla. It was saccular in shape and extended upward, inward and backward. The opening was

oval, measuring 15 mm. by 8 mm.; the longer axis was paralled with the folds of mucous membrane which at that point extended obliquely downward and outward. The sac measured 18 mm. deep. A small artery and vein crossed the outer wall of the sac and entered the intestinal wall at the base of the pouch.

The second diverticulum occurred immediately to the right of the bile papilla and extended upwards, outward and backward along the right side of the common bile duct. Its opening was a crescentic shaped slit which encircled the outer half of the ampulla. The common bile duct and the pancreatic duct opened independently into the duodenum; there was no ampulla of Vater. The diverticulum was saccular in shape and measured 15 mm. deep. It was empty, but pressure upon the gall bladder filled it with bile. A transverse fold of the mucous membrane hung down over the bile papilla and the opening of the diverticulum. No vessels were observed in the walls of this diverticulum.

The third diverticulum occurred immediately above the minor papilla. It was conical in shape and embedded in pancreatic tissue along the duct. It measured 8 mm. at the opening and 12 mm. deep. It was situated 18 mm. above the major papilla. A transverse fold of mucous membrane hung downward over it.

The muscularis ended abruptly at the opening of these diverticula and did not form a part of the sacs. The walls were represented by mucous membrane and muscularis mucosae.

The muscularis of the intestinal tract near the diverticula and at some distance from them was thin and showed considerable fatty degeneration. Fat was deposited in fine droplets throughout many muscle fibers. Sections were treated with potassium bichromate after the method of Bell,¹² to fix the fat, and paraffine sections were stained with Sharlach R.

The small diverticula of the transverse colon were likewise protrusions of the mucous membrane through the muscularis. No special relationship of blood vessels to these diverticula was established.

It is of interest to note the presence of marked chronic interstitial pancreatitis and slight periportal cirrhosis of the liver in

¹² Bell, E. T., *Jour. Path. and Bacteriology*, vol. 19, 1914, p. 105.

association with the diverticulum at the major papilla. Distention of this diverticulum would have exerted pressure on the common bile duct and pancreatic duct. Much significance, however, cannot be attached to this relation in view of the associated generalized sclerosis.

The two upper diverticula of the last case were obscured by folds of mucous membrane and were found only after special search was made. It seems probable that closer observation would show that diverticula of the duodenum occur more frequently than former observations have suggested.

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Resumen por G. Carl Huber, por el autor Tanzo Yoshinaga.

Contribución al estudio del desarrollo temprano del corazón de los mamíferos, con especial mención del del conejillo de Indias.

Las observaciones publicadas en el presente trabajo se basan en el estudio de una serie de estados muy próximos en el desarrollo de los embriones del conejillo de Indias, cortados en serie de 5 a 10 μ de espesor, tanto en el plano sagital como en el transverso. Los estados estudiados comprenden el periodo de desarrollo desde el duodécimo al décimo-quinto día después de la inseminación, o sea desde el momento en que aparecen por primera vez los angioblastos en la región más tarde ocupada por el corazón hasta el estado de corazón en forma de S. Los estados muy próximos han sido reconstruidos en placas de cera mediante el método de Born, y el estado más joven presenta el comienzo de la formación del espacio pericárdico.

Las pruebas obtenidas mediante este estudio son interpretadas por el autor como una demostración de que los angioblastos que han de formar el futuro corazón tubular endotelial derivan independientemente del mesodermo de la esplancopleura. El esbozo de los pliegues miocárdicos y su desarrollo progresivo son objeto de una discusión por parte del autor, quien presenta su desarrollo en una serie de figuras basadas en reconstrucciones.

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